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(54) Title: METHOD AND REAGENT FOR INHIBITING THE EXPRESSION OF DISEASE RELATED GENES				
<img alt="Diagram of a substrate RNA molecule showing four helices labeled Helix 1 through Helix 4. Helix 1 is at the bottom, Helix 2 is above it, Helix 3 is to the left of Helix 2, and Helix 4 is to the right of Helix 2. Nucleotides are represented by letters (A, G, C, T/U) and brackets [] indicating base pairing. Brackets are labeled with (N) subscripts (e.g., (N)1, (N)2, (N)3, (N)4, (N)5, (N)6, (N)7, (N)8, (N)9, (N)10, (N)11, (N)12, (N)13, (N)14, (N)15, (N)16, (N)17, (N)18, (N)19, (N)20, (N)21, (N)22, (N)23, (N)24, (N)25, (N)26, (N)27, (N)28, (N)29, (N)30, (N)31, (N)32, (N)33, (N)34, (N)35, (N)36, (N)37, (N)38, (N)39, (N)40, (N)41, (N)42, (N)43, (N)44, (N)45, (N)46, (N)47, (N)48, (N)49, (N)50, (N)51, (N)52, (N)53, (N)54, (N)55, (N)56, (N)57, (N)58, (N)59, (N)60, (N)61, (N)62, (N)63, (N)64, (N)65, (N)66, (N)67, (N)68, (N)69, (N)70, (N)71, (N)72, (N)73, (N)74, (N)75, (N)76, (N)77, (N)78, (N)79, (N)80, (N)81, (N)82, (N)83, (N)84, (N)85, (N)86, (N)87, (N)88, (N)89, (N)90, (N)91, (N)92, (N)93, (N)94, (N)95, (N)96, (N)97, (N)98, (N)99, (N)100, (N)101, (N)102, (N)103, (N)104, (N)105, (N)106, (N)107, (N)108, (N)109, (N)110, (N)111, (N)112, (N)113, (N)114, (N)115, (N)116, (N)117, (N)118, (N)119, (N)120, (N)121, (N)122, (N)123, (N)124, (N)125, (N)126, (N)127, (N)128, (N)129, (N)130, (N)131, (N)132, 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(57) Abstract

Enzymatic RNA molecules which cleave ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

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METHOD AND REAGENT FOR INHIBITING THE EXPRESSION
OF DISEASE RELATED GENESBackground of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

5

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl, and respiratory syncytial virus genes. Such ribozymes can be used in a method for treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ration of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.

Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF- α , p210bcr-abl, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.

Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF- α , p210bcr-abl, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF- α , p210bcr-abl, or RSV encoding mRNAs may be readily designed and are within the invention.

These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the 5 cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has 10 complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the 15 enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, 20 including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions. 25

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. 30 Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel et al., 1990, *Nucleic Acids Res.*, 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 35 16 of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell*, 35 849,

Neurospora VS RNA ribozyme motif is described by Collins (S ville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 *Proc. Natl. Acad. Sci. USA* 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799 Guo and Collins, 1995 *EMBO J.*, 14, 368) and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it has nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target (i.e., ICAM-1, IL-5, reLA, TNF- α , p210 bcr-abl or RSV proteins) encoding mRNA such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from vectors that are delivered to specific cells. By "vectors" is meant any nucleic acid and/or viral-based technique used to deliver a desired nucleic acid.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g. Scanlon, K.J. et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet, M., et al., 1992, *Antisense Res. Dev.*, 2, 3-15; Dropoulic, B., et al., 1992, *J. Virol.*, 66, 1432-41; Weerasinghe, M., et al., 1991, *J. Virol.*, 65, 5531-4; Ojwang, J.O., et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen C.J., et al., 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver, H., et al., 1990 *Science*, 247, 1222-1225). Those skilled in the art would realize that any ribozyme can be

expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their 5 totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser. 27, 15-6; Taira, K. et al., Nucleic Acids Res., 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res., 21, 3249-55, Chowrira et al., 1994 J. Biol. Chem., 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1, Rel A, IL-5, 10 TNF- α , p210bcr-abl or RSV encoding mRNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and 15 conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or 20 RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues 25 through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16, 30 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may 35 be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in 5 the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of 10 two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target 15 molecules and inhibit ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the 20 ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, 25 silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 30 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huiller et al., 1992 *EMBO J.* 11, 4411-8; Lisziewicz et al., 1993 *Proc. Natl. 35 Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from
5 the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead
10 ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead
ribozyme domain known in the art; Figure 2(b) is a diagrammatic
representation of the hammerhead ribozyme as divided by Uhlenbeck
15 (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure
2(c) is a similar diagram showing the hammerhead divided by Haseloff and
Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is
a similar diagram showing the hammerhead divided by Jeffries and
Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a
20 hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n
is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more
bases (preferably 3-20 bases, *i.e.*, m is from 1-20 or more). Helix 2 and
helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base).
Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20
25 base pairs) to stabilize the ribozyme structure, and preferably is a protein
binding site. In each instance, each N and N' independently is any normal
or modified base and each dash represents a potential base-pairing
interaction. These nucleotides may be modified at the sugar, base or
phosphate. Complete base-pairing is not required in the helices, but is
30 preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each
independently from 0 to any number, *e.g.* 20) as long as some base-pairing
is maintained. Essential bases are shown as specific bases in the
structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without 5 modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "—" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis 10 delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

15 Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

20 Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

25 Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

30 Figure 12 is a schematic representation of a one pot deprotection of RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCCAUCUC UUCCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phtalimido-nucleoside phosphoramidite.

Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *HindIII*-linearized plasmid. (23) HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 *supra*). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 *Nucleic Acids Res.* 20, 3252). Substitution of G₇₀ and A₇₁ to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 *Biochemistry* 29, 10695) and generates the HH(mutant) construct. (24) HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 *EMBO J* 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G₅₂ and U₇₇; HP(GC) has a Watson-Crick base pair between G₅₂ and C₇₇. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 *Nucleic Acids Res.* 21, 1991; Altschuler et al., 1992 *supra*). (25) HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 *Biochemistry* 31, 11843). The trans-ribozyme domain ends from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of 5 nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrotta & Been, 1991 *Nature* 350, 434). The ΔHDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

10 Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. H, Plasmid templates linearized with *Hind*III restriction enzyme. Transcripts from H templates contain four non-ribozyme 15 nucleotides at the 3' end. N, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from N templates contain 220 non-ribozyme nucleotides at the 3' end. R, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from R templates contain 450 non-ribozyme nucleotides at the 3' end.

20 Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 *Biochemistry* 29, 10159). HH+2, HH+37, and HH+52 are trans-acting ribozymes 25 produced by transcription from the HH, ΔHDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used 30 because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes 35 contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated with MgCl₂ (+) or with DEPC-treated water (-) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequence specific primer-extension assay. Solid arrows indicate the location of primer extension bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31, 10 shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, 15 refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

20 Figs. 33a-e Sequence of the primary tRNA_i^{met} and Δ3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This 25 modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ3-5 RNA. Δ3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ3-5 RNA; S3- a stable stem-loop structure was 30 incorporated at the 3' end of the Δ3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' region of the same RNA; S35Plus- in addition to 35 structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the Δ3-5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with Δ3-5 vectors. 35) Δ3-5/HHI and its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis 5 was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 10 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), 15 S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with Δ3-5 constructs described in Figs. 35 and 36. In a standard ribozyme cleavage reaction, 5 µg total RNA and trace amounts of 5' terminus-labeled ribozyme 20 target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, 25 following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by 30 spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the 35 pooled cells transduced with S35 construct. tRNA_imet, refers to the

endogenous tRNA. S35, r refers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a diagrammatic representation of 5T construct. In this construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T construct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. 25 The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. 30 A hairpin ribozyme targeted to site I is cloned into the indicated region of TRZ-tRNA chimera.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HHI hammerhead ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM 5 cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors; lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and adenoviruses vectors for ribozyme delivery. Both vectors utilize one or more ribozyme encoding transcription units (RZ) based on RNA polymerase II or 10 RNA polymerase III promoters. A. Diagram of an AAV-based vector containing minimal AAV sequences comprising the inverted terminal repeats (ITR) at each end of the vector genome, an optional selectable marker (Neo) driven by an exogenous promoter (Pro), a ribozyme transcription unit, and sufficient additional sequences (stuffer) to maintain a 15 vector length suitable for efficient packaging. B. Diagram of ribozyme expressing adenovirus vectors containing deletions of one or more wild type adenovirus coding regions (cross-hatched boxes marked as E1, pIX, E3, and E4), and insertion of the ribozyme transcription unit at any or several of those regions of deletions.

Fig. 58 is a graph showing the effect of arm length variation on the 20 activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6, 7/7, 8/8 and so on refers to the number of base-pairs being formed between the ribozyme and the target. For example, 5/8 means that the HH ribozyme forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a 25 total of 13 bp. -ΔG refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 *Ann. Rev. Biophys. Chem.* 17, 167). RPI A is a HH ribozyme with 6/6 binding arms.

Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by 30 ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme (HH-H) targeted against a site termed H. Variants of HH-H are also shown that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

Figs. 65a-b is a schematic representation of a method of this invention

5 to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular
10 helices formed between the ribozyme and the substrate. H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

15 Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme •substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO J.* 12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowira and Burke, 1992 *Nucleic Acids Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2.
20 Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramidite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

25

30 Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2 (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α -32P]CTP (Chowrima & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for *trans*-ribozyme cleavage reactions, a 2 kB region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above.

Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described above except that 20°C and at 26°C temperatures were used.

Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein q is ≥ 2 bases. Nomenclature is same as described under figure 3. C) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 103" L", wherein L is a non-nucleotide linker molecule (Benseler et al., 1993 J. Am. Chem. Soc. 115, 8483; Jennings et al., WO 94/13688). Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by non-nucleotide linker molecule "L" (Benseler et al., 1993 *supra*; Jennings et al., *supra*). Nomenclature is same as described under figure 3.

Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region "s" at the indicated location, wherein s is ≥ 1 base. Hairpin ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

Figs. 75a-e shows the structures of the 5'-C-alkyl-modified nucleotides. R₁ is as defined above. R is OH, H, O-protecting group, NH, or any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-C-alkyl-D-allose nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-C-alkyl-L-talose nucleosides and their phosphoramidites.

5 Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

10 Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

15 Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

20 Figure 84 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

25 Figure 86 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidine uridine, 2'-C-methoxycarboxymethylidine uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

5 Figure 89 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

10 Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

15 Figure 94 is a diagrammatic representation of a hammerhead ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is indicated.

20 Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

25 Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

30 Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are general schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used
5 in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection
of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop
complex. In addition, it indicates the location at which ligands can be
10 provided to target the R-loop complex to cells using at least three different
procedures, such as ligand receptor interaction, lipid or calcium phosphate
mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to
generate therapeutic ribozymes of unit length. This method is essentially
15 described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate,
carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A,
TNF- α , p210bcr-abl, or RSV genes expression and can be used to treat
20 diseases or diagnose such diseases. Ribozymes will be delivered to cells
in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1,
IL-5, rel A, TNF- α , p210bcr-abl, or RSV mRNA in these systems may prevent
or alleviate disease symptoms or conditions.

I. Target sites

25 Targets for useful ribozymes can be determined as disclosed in
Draper et al PCT WO93/23509, Sullivan et al., PCT WO94/02595 as well
as by Draper et al., PCT/US94/13129 and hereby incorporated by
reference herein in totality. Rather than repeat the guidance provided in
those documents here, below are provided specific examples of such
30 methods, not limiting to those in the art. Ribozymes to such targets are
designed as described in those applications and synthesized to be tested
in vitro and *in vivo*, as also described. Such ribozymes can also be

optimized and delivered as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for 5 targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are 10 individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm 15 lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides 20 representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is 25 added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozyme sites are chosen as the 30 most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used 35 follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yeilds are >98%. Inactive ribozymes are synthesized by substituting a U for G5 and a 5 U for A14 (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and 10 Uhlenbach, 1989, *Methods Enzymol.*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using heneral methods or are purified by 15 high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have 20 the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for 25 understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several 30 immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).

ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ -interferon, tumor necrosis factor- α , or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic cells) in a variety of tissues express high levels of ICAM-1 on their surface (Springer et al. *supra*; Dustin et al., *supra*; and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons et al., *supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.

ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd, 1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd et al., *supra*). The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.

By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro*.

5 The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences
10 are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

15 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be
25 monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNase protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

30 As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft
35 rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene 5 construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection 10 and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and 15 arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the 20 role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This 25 list is not meant to be complete and those in the art will recognize further conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation* 30 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

5 ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

10 Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

15 Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

20 Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

25 In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegner et al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethasone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

5 Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993 *J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989 *Lancet* 2, 1298-302).

10 Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992 *Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Arerugi* 41, 1507-14).

15 Circulating LFA-1⁺ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993 *Scand J Immunol* 37, 377-80).

Example 2: IL-5

Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g., by inhibiting the synthesis 20 of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF- α , gamma interferon, VCAM, ILAM-1, 25 ELAM-1 and NF- κ B. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF- α R on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain 30 neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gene-related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 supra; Garssen et al., 1991 Am. Rev. Respir. Dis. 144, 931-938; Larsen et al., 1992 J. Clin. Invest. 89, 747-752; Mauser et al., 1993 supra). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cleave IL-5 target sequences *in vitro* is evaluated.

The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.

Uses

Interleukin 5 (IL-5), a cytokine produced by CD4+ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by Takatsu et al., 1988 *Immunol. Rev.* 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 *Blood* 73, 1504-12), vascular adhesion (Walsh et al., 1990 *Immunology* 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al., 1988 *J. Exp. Med.* 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 *J. Exp. Med.* 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

Several studies have shown a direct correlation between the number of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 *J. Investig. Allergol. Clin. Immunol.* 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for eosinophils, activated T helper cells and recovery of pulmonary function over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

Bronchoalveolar lavage cells were screened for production of cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic 5 patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was 10 observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after 15 allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or 20 mast cell subtypes were observed. The number of cells expressing IL-5 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from 25 asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for 30 eosinophilia and the responsiveness to the bronchoconstriction substance P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mauser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with 35 monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge
5 (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studies indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-5 a good candidate for target selection. The antibody studies neutralized
10 IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-derived factors like IL-5 are responsible for the activation and maintenance of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.
15

Atopy – is characterized by the development of type I hypersensitive reactions associated with exposure to certain environmental antigens. One of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and
25 Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for example, can lead to blood, peritoneal and/or tissue eosinophilia, all of
30 which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Tryptophan-associated eosinophilia-myalgia syndrome (EMS)- The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 *J Invest. Dermatol.* 100, 97s). Pathologic 5 and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 *supra*) by activating eosinophils and other inflammatory cells.

10 Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5 function is described above; available cellular and activity assays are 15 numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 *supra*) and can be used to optimize activity.

Example 3: NF- κ B

20 Ribozymes that cleave *rel A* mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor- α (TNF- α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by 25 the transcriptional regulator, NF- κ B. One subunit of NF- κ B, the *relA* gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by *rel A* or 30 TNF- α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF- κ B, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF- κ B now is known to activate transcription of a variety of other 35 cellular genes (e.g., cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (e.g., phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF- κ B has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each 5 subunit bears a stretch of 300 amino acids that is homologous to the oncogene, *v-rel*. The activity first described as NF- κ B is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF- κ B (encoded by the nf- κ B2 or nf- κ B1 genes, respectively) are generated from the precursors NF- κ B1 (p105) or NF- κ B2 (p100). The p65 subunit of NF- κ B (now 10 termed Rel A) is encoded by the *rel A* locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci USA 89, 1529-1533). For instance, the heterodimer of NF- κ B1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, 15 VCAM-1, while NF- κ B2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., Mol. Cell. Biol. 13, 6283-6289 (1993)). Conversely, heterodimers of NF- κ B2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF- κ B1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. 20 Nabel, J. Virol. 1992 66, 3883-3887). Similarly, blocking *rel A* gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF- κ B1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially 25 assigned to NF- κ B in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the *rel* family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential 30 transcriptional activation functions can be performed by more than one member of the *rel* family.

A number of specific inhibitors of NF- κ B function in cells exist, including treatment with phosphorothioate antisense oligonucleotide, treatment with double-stranded NF- κ B binding sites, and over expression 35 of the natural inhibitor MAD-3 (an I κ B family member). These agents have

been used to show that NF- κ B is required for induction of a number of molecules involved in inflammation, as described below.

•NF- κ B is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., *Science* 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 5 *1993 Mol. Cell. Biol.* 13, 6137-46).

•NF- κ B is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 *Mol. Cell. Biol.* 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 *J. Exp. Med.* 179, 503-512) on endothelial cells.

10 •NF- κ B is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

The above studies suggest that NF- κ B is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF- κ B and 15 inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF- κ B. The glucocorticoid receptor and p65 both act at NF- κ B binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 *J. Biol. Chem.* 269, 6185-6192). Glucocorticoid receptor inhibits NF- κ B-mediated induction of IL-6 (Ray and Prefontaine, 1994 *Proc. Natl Acad. Sci USA* 91, 20 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*).

Ribozymes of this invention block to some extent NF- κ B expression 25 and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

30 The sequence of human and mouse *relA* mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that 5 these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

10 By engineering ribozyme motifs we have designed several ribozymes directed against *relA* mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *relA* target sequences *in vitro* is evaluated.

15 The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS 20 analysis. *Rel A* mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Activity of NF- κ B will be monitored by gel-retardation assays. Ribozymes that block the induction of NF- κ B activity and/or *relA* mRNA by more than 50% will be identified.

25 RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*relA* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with 30 *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*relA* ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate 35 inflammatory and immune responses in these diseases.

Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the 5 treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

10 •Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the 15 synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, *J. Clin. Invest.* 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple 20 administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Restenosis.

25 Expression of NF- κ B in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF- κ B is required for the expression of the oncogene c-myb (F.A. La Rosa, J.W. Pierce, G.E. 30 Soneneschein, *Mol. Cell. Biol.* 14, 1039-44 (1994)). Thus NF- κ B induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

•Transplantation.

NF- κ B is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., *J. Clin. Invest.* 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are
5 treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF- κ B in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the
10 treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 and B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

15 •Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory
20 mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer
25 techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette
30 that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF- κ B activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF- κ B

function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for NF- κ B function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

5 Example 4: TNF- α

Ribozymes that cleave the specific sites in TNF- α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

10 Tumor necrosis factor- α (TNF- α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of TNF- α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

15 TNF- α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old, 1985 *Science* 230, 4225-4231). TNF- α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 *Nature* 316, 552-554). The cDNA and the genomic locus for TNF- α have been cloned and found to be related to TNF- β (Shakhov et al., 1990 20 *J. Exp. Med.* 171, 35-47). Both TNF- α and TNF- β bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 *Science* 248, 1019-1023). TNF- α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine 25 activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turetskaya et al., 1991 in Tumor Necrosis Factor: Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF- α is regulated transcriptionally and 30 translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 *Cell* 53, 45-53). Once secreted, the serum half life of TNF- α is approximately 30 minutes. The tight regulation of TNF- α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF- α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF- α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in SanDiego, CA; and "Development of anti-TNF- α ribozymes for the control of TNF- α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNF α targeted ribozymes.

Ribozymes of this invention block to some extent TNF- α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF- α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF- α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF- α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of 5 two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV.

In a preferred embodiment of the invention, a transcription unit 10 expressing a ribozyme that cleaves TNF- α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retrovirus vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, I-473.; Nabel et al., 1990 Science, 249, 1285-1288) and 15 both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an 20 injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo*.

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

25 By engineering ribozyme motifs we have designed several ribozymes directed against TNF- α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF- α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing 30 bacterial lipopolysaccharide (LPS)-induced TNF- α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF- α expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. TNF- α mRNA levels will be 35 assessed by Northern analysis, RNase protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF- α activity and/or TNF- α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF- α secretion are selected. The TNF- α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF- α ribozymes to block TNF- α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF- α ribozymes after induction by the injection of fixed *Streptococcus*.

To examine the effect of anti-TNF- α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF- α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF- α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adenovirus associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF- α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57bl/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at 2.5×10^5 /well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

5 The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial 10 lipopolysaccharide (LPS) was added to each well to stimulate TNF production.

Quantitation of TNF- α in mouse macrophages:

Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70°C. Quantitation of TNF- α was done by a 15 specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF- α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF- α containing supernatants. TNF- α was then detected using a murine TNF- α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled 20 to alkaline phosphatase.

Assessment of reagent toxicity:

Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium 25 bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.

Uses

30 The association between TNF- α and bacterial sepsis, rheumatoid arthritis, and autoimmune disease make TNF- α an attractive target for therapeutic intervention [Tracy & Cerami 1992 supra; Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788; Jacob, 1992 J. Autoimmun. 5 (Supp. A), 133-143].

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 5 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of 10 pro-inflammatory cytokines which are not normally detected in the serum, such as TNF- α , interleukin-1 β (IL-1 β), γ -interferon (IFN- γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as 15 leukotriene b4, prostaglandin E2, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF- α is detected early in the course of septic shock in a large fraction 20 of patients (de Boer et al., 1992 supra). In animal models, injection of TNF- α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1 β , IL-6, or IL-8 does not induce shock. Injection of TNF- α 25 also causes an elevation of IL-1 β , IL-6, IL-8, PgE₂, acute phase proteins, and TxA₂ in the serum of experimental animals (de Boer et al., 1992 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF- α antibodies. The cumulative evidence indicates that TNF- α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

30 Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to 35 contain elevated levels of TNF- α , IL-1 α and IL-1 β , IL-6, GM-CSF, and TGF-

β (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF- α to these cultures has been shown to reduce IL-1 α/β production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF- α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF- β , has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF- α , IL-1 α/β , and IL-6 from macrophages near the cartilage/pannus junction when the pannus is invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF- α and TGF- β have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF- α has also been shown to increase osteoclast activity and bone resorption, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).

Elimination of TNF- α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1 α/β , IL-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF- α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

Treatment with an anti-TNF- α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF- α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, 5 neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated CD4⁺ cells of the T_H-1 phenotype, although some CD8⁺ and CD4⁺/CD8⁺ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of 10 psoriasis including auto-antibodies and auto-reactive T-cells, overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 15 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF- α , IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, IFN- γ , and TNF- α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, 20 and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF- α , IL-6, and TNF- α , which could increase proliferation in an autocrine fashion (Oxholm 25 et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1 α , IL-1 β , IL-6, IL-8, TNF- α . These factors 30 activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF- α , IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF- α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through

5 the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the $T_H\cdot 1$ phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN- γ secreted by the T-cells synergizes with the TNF- α from dermal dendrocytes to increase

10 keratinocyte proliferation and the levels of TGF- α , IL-8, and IL-6 production. IFN- γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production

15 by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are

20 TNF- α expression by the dermal dendrocyte to maintain activated endothelium and keratinocytes, and IFN- γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX

25 (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns.

30 Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for

35 treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these 5 treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

HIV and AIDS

The human immunodeficiency virus (HIV) causes several 10 fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF- α and TNF- β levels, hypergammaglobulinemia, and lymphoma/leukemia 15 (Rosenberg & Fauci, 1990 *Immun. Today* 11, 176; Weiss 1993 *Science* 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS 20 suggests that some of the pathology may be due to cytokine dysregulation.

Levels of serum TNF- α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 *supra*). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF- α and IL-6. This response has been reproduced using purified gp120, 25 the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 *J. Virol.* 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF- α and IL-6 may be an adaptive mechanism of the virus. TNF- α has been shown to upregulate transcription 30 of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF- α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing 35 virus production from latently infected cells and by driving replication of the virus in newly infected cells.

The role of TNF- α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF- α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF- α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF- α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF- α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF- α . Thus, levels of secreted TNF- α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.

Chronic elevation of TNF- α has been shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF- α frequently observed in AIDS patients. Similarly, TNF- α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J Immunol 149, 3727).

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF- α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

5 Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several
10 months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus
15 vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

20 The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum corneum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 Supra).
25 Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

30 •Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

5 Thus, ribozymes of the present invention that cleave TNF- α mRNA and thereby TNF- α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF- α function is described above; available cellular and activity assays
10 are number, reproducible, and accurate. Animal models for TNF- α function and for each of the suggested disease targets exist and can be used to optimize activity.

Example 5: p210bcr-abl

15 Chronic myelogenous leukemia exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (*i.e.*, the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This
20 lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (*e.g.* approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients
25 which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 *Br. J. Haematol.* 69, 239).

30 The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, *Cancer Genet. Cytogenet.* 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2 junction) from the major breakpoint cluster region of the *bcr* gene is spliced
35

to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259; 5 Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

The b3-a2 and b2-a2 fusion mRNAs encode 210 kd *bcr-abl* fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the *bcr-abl* fusion protein (*p210^{bcr-abl}*) in the evolution and maintenance of the 10 leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of *p210^{bcr-abl}* expression. These inhibitory molecules have been shown to inhibit the in vitro proliferation of leukemic cells in bone marrow from CML patients. Szczylak et al., 1991 Science 253, 562).

15 Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome, 20 specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCCU-3'.

Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective 25 to cleave oncogenic variants of H-ras RNA. This ribozyme is said to inhibit H-ras expression in response to external stimuli.

The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML. 30 Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either in vivo administration to reduce the tumor burden, or ex vivo treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl*/mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 *supra*) is an *in vitro* transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only *ex vivo* treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210*bcr-abl* expression and can be used to treat disease or diagnose such disease.

5 Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr/abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

The sequence of human *bcr/abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA 10 that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

15 The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of 20 hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the 25 ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro*.

30 The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210*bcr-abl* protein and mRNA by more than 20% are identified.

5 Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified
10 under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in *Virology* ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of
15 capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear
20 compartment (Hall, 1990 in *Principles and Practice of Infectious Diseases* ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)]
25 found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used
30 with multiple transcription initiation sites (Barik et al., 1992 J. Virol. 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang et al., 1985 Virus Res. 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are
35 much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci. USA* 84, 5625).

Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, *Mmwr Morb Mortal Wkly Rep.*, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation .

Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modern intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can 5 provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristam *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*, 10 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the 15 use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment 20 period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious 25 symptoms or for patients with underlying bronchopulmonary or cardiac disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY]. Since 30 ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

35 Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytial virus.

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The 5 invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the NS1 (1C), NS2 (1B) and N viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).

10 Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described 15 that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (P, M, SH, G, F, 22K and L) and the genomic RNA may be readily 20 designed and are within the invention.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these 25 Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

30 Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviate disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 5 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson *et al.*, 1987 *supra*). RSV proteins 1C, 1B and N 10 are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding 15 algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

20 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et* 25 *al.*, 1987 *J. Am. Chem. Soc.*, 109, 7845-7854 and in Scaringe *et al.*, 1990 *Nucleic Acids Res.*, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for 30 G5 and a U for A14 (numbering from Hertel *et al.*, 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified 35 extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

5 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
10 hammerhead ribozymes listed in Tables 32 and 34 (5'-GGCCGAAAGGCC-
3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution,
15 deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

25 Numerous common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA
30 assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNase protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not be repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing 5 ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergrén, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and 10 Rossi et al., International Publication No. WO 91/03162, as well as Jennings et al., WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of 15 stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to 20 those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. 25 Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., 30 supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II 35 (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells

5 (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U.S.A., 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet

10 et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U.S.A., 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U.S.A., 90, 6340-4; L'Huillier et al., 1992 EMBO J., 11, 4411-8; Lisziewicz et al., 1993 Proc. Natl. Acad. Sci. U.S.A., 90, 8000-4).

15 The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992 Eur. J. Biochem. 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, e.g., through the

20 use of a catheter, stent or infusion pump.

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Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

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using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role
5 (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies
10 (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art,
15 and include detection of the presence of mRNA associated with ICAM-1, relA, TNF- α , p210, bcr-abl or RSV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second
20 ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and
25 cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype
30 (i.e., ICAM-1, rel A, TNF α , p210bcr-abl or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

5 There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation.

10 Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and

15 purification procedure of the resulting ribozyme be used.

To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH₃/EtOH (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al.* *Nucleic Acids Res.* 35 1990, 18, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedure in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, e.g. Na⁺, Li⁺ etc. A final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41) improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-S-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an
5 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the
10 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.
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30 2. Using 5-S-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

35 3. Using alkylamine (MA, where alkyl is preferably methyl, ethyl, propyl or butyl) or NH₄OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (vs 4-20 h @ 55-65 °C using NH₄OH/EtOH or NH₃/EtOH, *vide supra*). Other alkylamines, e.g. ethylamine, propylamine, butylamine etc. may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride (aHF•TEA)
5 @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group
(vs 8 - 24 h using TBAF, *vide supra* or TEA•3HF for 24 h (Gasparutto *et al.* *Nucleic Acids Res.* 1992, 20, 5159-5166). Other alkylamine•HF complexes may also be used, e.g. trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the
10 fully deprotected RNA. These resins include, but are not limited to,
quaternary or tertiary amino derivatized stationary phases such as silica or
polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-
Q®.

Thus, the invention features an improved method for the coupling of
15 RNA phosphoramidites; for the removal of amide or substituted amide
protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting
groups. Such methods enhance the production of RNA or analogs of the
type described above (e.g., with substituted 2'-groups), and allow efficient
20 synthesis of large amounts of such RNA. Such RNA may also have
enzymatic activity and be purified without loss of that activity. While specific
examples are given herein, those in the art will recognize that equivalent
chemical reactions can be performed with the alternative chemicals noted
above, which can be optimized and selected by routine experimentation.

In another aspect, the invention features an improved method for the
25 purification or analysis of RNA or enzymatic RNA molecules (e.g. 28-70
nucleotides in length) by passing said RNA or enzymatic RNA molecule
over an HPLC, e.g., reverse phase and/or an anion exchange
chromatography column. The method of purification improves the catalytic
activity of enzymatic RNAs over the gel purification method (see Figure 10).

30 Draper *et al.*, PCT WO93/23569, incorporated by reference herein,
disclosed reverse phase HPLC purification. The purification of long RNA
molecules may be accomplished using anion exchange chromatography,
particularly in conjunction with alkali perchlorate salts. This system may be
used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100[®] or a Pharmacia Mono Q[®] anion exchange column for the purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the 5 formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, e.g., lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step 10 is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, e.g. polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled 15 and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an 20 enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA 25 molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having 30 a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 µm, preferably 5 µm.

Activation

The synthesis of RNA molecules may be accomplished chemically or 35 enzymatically. In the case of chemical synthesis the use of tetrazole as an activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. 1987, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-S-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-S-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987*supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-S-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 μ mol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-O-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 μ mol) of phosphoramidite and a forty-five-fold excess of S-ethyl tetrazole (4.5 mL of

0.25 M = 1125 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 5 2% DCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up 10 from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH₄OH/EtOH:3/1 (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-15 7854) or NH₃/EtOH (Scaringe *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341) for -20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH₄OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH₄OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH₄OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the 25 synthesis column to a 4 mL glass screw top vial. NH₄OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL 30 of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements 35 outlined in this application for base deprotection.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM NaClO₄). A gradient from 180-210 mM NaClO₄ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM NaClO₄ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.

For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H₂O to lower the salt concentration and applied to a Pharmacia Mono Q® 16/10 column. A 5 gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 85% full length material 10 were pooled. The pool was applied to a Pharmacia RPC® column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow 15 column. The column was thoroughly washed with 20 mM NH₄CO₃H/10% CH₃CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH₄CO₃H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource 20 RPC column. A gradient from 20-55% B (20 mM NH₄CO₃H/25% CH₃CN, buffer A = 20 mM NH₄CO₃H/10% CH₃CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 60% full length 25 material were pooled. The pooled fractions were then submitted to manual detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H₂O, dried down and resuspended in H₂O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac® column. The material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

30 Example 11 Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 μM, 200 nM, 40 nM or 8 nM and the final substrate RNA 35 concentrations were ~ 1 nM. Total reaction volumes were 50 μL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were

initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 μ L were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were 5 performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely 10 prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 μ mol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify 15 the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent. 20 The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramidite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by 25 either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from 30 Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

The two sulfurizing reagents that have been used to synthesize ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem.*).

15 The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during previous synthesis.

A major improvement is the use of an activating agent, 5-S-ethyltetrazole or 5-S-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosphorothioate, the iodine solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 min coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite

and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: ASE = (PS/Total)^{1/n-1}

where, PS = integrated ³¹P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phtalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with N-(9-fluorenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pieken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH₃CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17,
5 phosphoramidite 17 was synthesized starting from 2'-deoxy-2'-aminonucleoside (12) using transient protection with Markevich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate 13 was obtained in 50% yield, however subsequent introduction of N-phtaloyl (Pht) group by
10 Nefken's method (Nefkens, 1960 *Nature* 185, 306), desilylation (15), dimethoxytrytilation (16) and phosphorylation led to phosphoramidite 17. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phtaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05
15 equivalents of Nefkens reagent in DMF overnight with subsequent treatment with Et₃N (1 hour) only 10-15% of N and 5'(3')-bis-phtaloyl derivatives were formed with the major component being N-Pht-derivative 15. The N,O-bis by-products could be selectively and quantitatively converted to N-Pht derivative 15 by treatment of crude reaction mixture
20 with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate 16 involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCI/Et₃N and resulting in the preparation of DMT derivative 16 in 85% overall yield as follows. Standard phosphorylation of
25 16 produced phosphoramidite 17 in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (Dmf) and dried in vacuo overnight. 50 mls of Aldrich sure-seal Dmf was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes
30 to produce a clear solution. 1.0 grams (1.05 eq.) of N-carbethoxyphthalimide (Nefken's reagent, 98% Jannsen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in CHCl₃) and 57 µl of TEA (0.1 eq.) was added to effect closure of the
35 phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

(Lancast r Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted 5 with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ^1H NMR). Phosphoramidites were then prepared using standard protocols described above.

10 With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were either protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base- 15 composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.

Protecting 2' Position with a SEM Group

20 There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the 25 same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441). However, long exposure times to tetra-*n*- 30 butylammonium fluoride (TBAF) are generally required to fully remove this protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic 35 ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990,

18, 5433-5441 and Stawinski,J.; Stromberg,R.; Thelin,M.; Westman,E. *Nucleic Acids Res.* 1988, 16, 9285-9298).

The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harsh st acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $\text{BF}_3\text{-OEt}_2$ very quickly.

There follows a method for synthesis of RNA by protecting the 2'-position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, *International Publication No. WO 92/07065*, Perrault *et al.*, *Nature* 1990, 344, 565-568, Pieken *et al.*, *Science* 1991, 253, 314-317, Usman,N.; Cedergren,R.J. *Trends in Biochem. Sci.* 1992, 17, 334-339, Usman *et al.*, PCT WO93/15187, and Sproat,B. *European Patent Application 92110298.4*.

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions. In a preferred embodiment, the conditions are dibutyltin oxide, tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride etherate ($\text{BF}_3\text{-OEt}_2$) under SEM removing conditions, e.g., in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-O-SEM protected nucleosides and phosphoramidites. Briefly, a 5'-protected nucleoside (1) is protected at the 2'- or 3'-position by contacting with a derivative of SEM under appropriate conditions. Specifically, those conditions include contacting the nucleoside with dibutyltin oxide and SEM chloride. The 2 regioisomers are separated by chromatography and the 2'-protected moiety is converted into a phosphoramidite by standard procedure. The 3'-protected nucleoside is converted into a succinate derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl ethers is shown. This contrasts with the method shown in Figure 21 in which deprotection of RNA containing an SEM group is performed. In step 1, the base protecting groups and cyanoethyl groups are removed by standard procedure. The SEM group is then removed as shown in the Figure. The details of the synthesis of phosphoramidites and SEM protected nucleosides and their use in synthesis of oligonucleotides and subsequent deprotection of

Example 14: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine (2)

Referring to Figure 19, 5'-O-dimethoxytrityl uridine 1 (1.0 g, 1.83 mmol) in CH₃CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT (about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight and then filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected nucleoside 2 and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside 3.

Example 15: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside 2 was detritylated following standard methods, as shown in Figure 19.

Example 16: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5',3'-O-Acetyl Uridine (5)

Nucleoside 4 was acetylated following standard methods, as shown in Figure 19.

5 **Example 17: Synthesis of 5',3'-O-Acetyl Uridine (6)**

Referring to Figure 19, the fully protected uridine 5 (32 mg, 0.07 mmol) was dissolved in CH₃CN (700 μL) and BF₃•OEt₂ (17.5 μL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH₂Cl₂) gave 10 20 mg (88%) of SEM deprotected nucleoside 6.

Example 18: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-3'-O-Succinyl-5'-O-Dimethoxytrityl Uridine (2)

Nucleoside 3 was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

15 **Example 19: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (8)**

Nucleoside 3 was phosphorylated following standard methods, as shown in Figure 19.

20 **Example 20: Synthesis of RNA Using 2'-O-SEM Protection**

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 25 1990, 18, 5433-5441. The phosphoramidite 8 was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 μL of 0.1 M = 32.5 μmol) of phosphoramidite and a 80-fold excess of tetrazole 30 (400 μL of 0.5 M = 200 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synth sis Grade acetonitrile was used directly from the reagent bottle.

5 Referring to Figure 21, the homopolymer was base deprotected with NH₃/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H₂O:CH₃CN:MeOH. The combined solutions were dried down and then diluted with CH₃CN (1 mL). BF₃•OEt₂ (2.5 µL, 30 µmol) was added to the solution and aliquots were removed at
10 ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In
15 general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript produced from the vector by only one other ribozyme. The system is useful
20 for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow productiuon of large amounts of a desired riboqyne. The vectors of
25 this invention allow a higher yield synthesis of a ribozyme in the form of an RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-acting or desired ribozyme instead of processing only one end, or only one
30 ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as
5 described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use
10 isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility
15 studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme,
20 or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a
25 bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagemid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-
30 directed homologous recombination into viral or viroid RNA genomes. Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin, hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of the second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several different vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector; and the vector is chosen from a plasmid, 5 cosmid, phagemid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability 10 of the RNA produced by the vector; and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction 15 of the vector.

In a second aspect, the invention features a method for formation of a 20 ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, 25 such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

30 In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

35 In preferred embodiments, three different ribozyme motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined sequences that rapidly self-cleave *in vitro* (Symons, 1992 *Annu. Rev. Biochem.*, 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs self-process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 *supra*; and Altschuler et al., 1992 *Gene* 122, 85. The present invention enables the use of *cis*-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism 15 or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, *id.*, or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or 25 double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picornaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

30 .. In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs—the hammerhead, the hairpin and the hepatitis delta virus ribozyme—in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the 35 ribozyme-containing transcripts to be as similar as possible. To this end,

all the ribozyme cassettes contained the same trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only.

5 Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.

10 A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated by a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that 15 other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known 20 in the art.

The trans-acting ribozyme used in this study is targeted to a site B (5'...CUGGAGUC_↓GACCUUC...3'). The 5' binding arm of the ribozyme, 5'-GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving 25 ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans-cleaving ribozyme once cis-cleavage occurs. The only differences 30 between the constructs lie in the 3' binding arm of the ribozyme, where 35

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

The cis-cleaving hammerhead ribozyme used in the HH cassette is
5 based on the design of Grosshans and Cech, 1991 supra. As shown in
Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the
required base-pairing interactions of the cis-cleaving ribozyme to form stem
I. Two extra nucleotides, UC, were included at the end of the 3' binding
10 arm to form the self-processing hammerhead ribozyme site (Ruffner et al.,
1990 supra) which remain on the 3' end of the trans-acting ribozyme
following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is
based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A
tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link
15 the two portions and thus allows a minimal five nucleotides to remain at the
end of the released trans-acting ribozyme following self-processing. Two
variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was
constructed with a G-U wobble base pair in helix 2 (A52G substitution;
Figure 24). This slight destabilization of helix 2 was intended to improve
20 self-processing activity by promoting product release and preventing the
reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129;
Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was
constructed as a control for strong base-pairing interactions in helix 2
(U77C and A52G substitution; Figure 24). Another modification to
25 discourage the reverse ligation reaction of the hairpin ribozyme was to
shorten helix 1 (Figure 24) by one base pair relative to the wild-type
sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to
the cleavage site is a pyrimidine, and somewhat less so when adenosine is
30 in that position. No other sequence requirements have been identified
upstream of the cleavage site, however, we have observed some decrease
in activity when a stem-loop structure was present within 2 nt of the
cleavage site. The HDV self-processing construct (Fig 25) was designed to
generate the trans-acting hammerhead ribozyme with only two additional
35 nucleotides at its 3' end after self-processing. The HDV sequence used
here is based on the anti-genomic sequence (Perrotta & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (*Biochemistry* 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoR*1/*Hind*III-digested puc18 and transformed into *E. coli* strain DH5α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.

Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 µCi [γ -³²P]GTP, 200 µM each NTP and 0.5 to 1 µg of linearized plasmid template. The concentration of MgCl₂ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons, equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ -³²P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg²⁺ was included at 10 mM above the nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5
5 non-ribozyme nucleotides at the 3' end (*HindIII*-digested template), 220 nucleotides (*NdeI* digested templates) or 454 nucleotides of downstream sequence (*RcaI* digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides
10 essential for hammerhead ribozyme activity (Ruffner et al., 1990 *supra*) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present. Comparison of the amounts of released
15 trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition,
20 the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at
25 self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes
30 to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

HindIII-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris-HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 µM CTP; 40 µCi [α -32P]CTP; 12 mM MgCl₂; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/µl). Aliquots of 5 µl were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software, Reeding, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

where t represents time and k represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with HindIII so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (k) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min^{-1}) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme—as measured here during transcription—is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target in trans. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and ΔHDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical—with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and ΔHDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α -32P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager® (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the ΔHDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding
5 the target sequence. In contrast, the additional nucleotides at the end of ΔHDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the ΔHDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans-ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the
10 observed reductions in activity for the ΔHDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

15 **Example 25: RNA self-processing *in vivo***

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A
20 mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

25 Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with ~ 5x10⁵ cells/well. Cells were transfected with circular plasmids (5 µg/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis
30 buffer (200 µl/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with
35 an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for 5 min. in the absence of Mg²⁺, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris-HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM MgCl₂; 1 mM each dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTTCGAGCTT-3'; HDV primer, 5'-10 AAGTAGCCCAGGTGGACC-3'; HP primer, 5'-15 ACCAGGTAATATACCACAAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in 20 addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +MgCl₂" vs. "Cellular").

25 Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly 30 suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

35 Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent

metal ions such as Mg²⁺ and Ca²⁺ that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg²⁺ (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg²⁺ required for the self-processing reaction (Michel et al. 1992 *Genes & Dev.* 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (Figure 29, *in vitro* "-MgCl₂" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM MgCl₂ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (Figure 29, *in vitro* "+MgCl₂" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of the added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

10 In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as
15 described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes
20 by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a
25 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

30 Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), ad novirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickofer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

The construct described in this invention is able to accumulate RNA to a significantly higher level than other constructs, even those in which 5' and 3' ends are involved in hairpin loops. Using such a construct the level of expression of a foreign RNA can be increased to between 20,000 and 50,000 copies per cell. This makes such constructs, and the vectors encoding such constructs, excellent for use in decoy, therapeutic editing and antisense protocols as well as for ribozyme formation. In addition, the molecules can be used as agonist or antagonist RNAs (affinity RNAs). Generally, applicant believes that the intramolecular base-paired interaction between the 5' terminus and the 3' region of the RNA should be in a double-stranded structure in order to achieve enhanced RNA accumulation.

Thus, in one preferred embodiment the invention features a pol III promoter system (e.g., a type 2 system) used to synthesize a chimeric RNA molecule which includes tRNA sequences and a desired RNA (e.g., a tRNA-based molecule).

The following exemplifies this invention with a type 2 pol III promoter and a tRNA gene. Specifically to illustrate the broad invention, the RNA molecule in the following example has an A box and a B box of the type 2 pol III promoter system and has a 5' terminus or region able to base-pair with at least 8 bases of a complementary 3' end or region of the same RNA molecule. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using other pol III promoter systems and techniques generally known in the art.

By "terminus" is meant the terminal bases of an RNA molecule, ending in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a stretch of bases 5' or 3' from the terminus that are involved in base-paired interactions. It need not be adjacent to the end of the RNA. Applicant has determined that base pairing of at least one end of the RNA molecule with a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular bas-paired interaction with complementary 5 nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' 10 terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that 15 is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences 20 which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for a optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a 25 pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in 30 between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B 35 box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such 5 molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 *Annu. Rev. Biochem.* 60, 631-652). By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, 1988 *J.American. Med. Assoc.* 260, 3030-10 3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic 15 portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural 20 binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV 25 RNA (Sullenger et al., 1990 *Cell* 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind 30 to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, see Tsai et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 8864-8868).

5 In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

Thus, the invention features a transcribed non-naturally occurring RNA
10 molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

15 In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the
20 desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an
25 intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

In most preferred embodiments, the molecule is transcribed by a RNA
30 polymerase III based promoter system, e.g., a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51.

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by 5 introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is 10 a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.(USA)* 90, 6340-15 6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol 20 III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

25 Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk 30 treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNA_imet gene and termed Δ3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted 5 to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et 10 al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic 15 levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the Δ3-5 vector system (These constructs are termed "Δ3-5/HHI"; Fig. 34). On average, 20 ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the Δ3-5 chimera, the applicant made a series of modified Δ3-5 gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the 25 ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original Δ3-5/HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those 30 achieved with the original Δ3-5/HHI version of this vector.

.. The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such 35 as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

5 As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of
10 expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

A3-5 Vectors

15 The use of a truncated human tRNA_imet gene, termed Δ3-5 (*Fig. 33*; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras
20 containing tRNA_imet sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

25 Since the Δ3-5 vector combination has been successfully used to express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as "Δ3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (*Fig. 35*). To try and improve accumulation of the
30 ribozyme, applicant incorporated various RNA structural elements (*Fig. 34*) into one of the ribozyme chimeras (Δ3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degradation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences 5 can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the tRNA_imet domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the A3-5 chimeras (Figure 34). These stem-loop structures are 10 also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol.* 15 *Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A 20 and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a 25 desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of A3-5-Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 30 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTG 3' and 5' CGCGTCAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 µM each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-stranded molecule using Sequenase® enzyme (US Biochemicals) in a 35

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM MgCl₂, 50 mM NaCl, 0.5 mM each of the four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

5 The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*HI and *Mlu*I) to generate ends that were suitable for cloning into the Δ3-5 vector.

10 The double-stranded insert DNA was ligated to the Δ3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM MgCl₂, 10 mM DTT, 0.066 μM ATP and 0.1U/μl T4 DNA Ligase (US Biochemicals).

15 Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

20 Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase® DNA sequencing kit (US Biochemicals).

25 The resulting recombinant Δ3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ3-5-S35 containing vector using *Sac*II and *Bam*HI restriction sites.

Example 27: Northern analysis

RNA from the transduced MT2 cells were extracted and the presence of Δ3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). 30 Northern analysis of RNA extracted from MT2 transductants showed that Δ3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35.36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35.36). The pattern of

expression seen from the Δ3-5/HHI ribozyme chimera was similar to 12 other ribozymes cloned into the Δ3-5 vector (not shown). In MT-2 cell line, Δ3-5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

5 Addition of a stem-loop onto the 3' end of Δ3-5/HHI did not lead to increased Δ3-5 levels (S3 in Fig. 35,36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35,36).

10 Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original Δ3-5/HHI vector transcripts (Fig. 35,36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

15 To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

25 Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39).
30 All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme levels among the clones ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

5 The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original Δ3-5 vector. Therefore, the S35 gene unit should be much more effective
10 in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

Finally, the bulk S35-transduced line, resistant to G418, was propagated for a period of 3 months (in the absence of G418) to determine
15 if ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propagate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme
20 expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

A transcription unit, termed TRZ, is designed that contains the S35 motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I
30 (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme transcripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-
5 tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily
10 generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme
15 expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin selectable marker and a ribozyme (S35/HHI) expressed from pol III met; tRNA-driven promoter. Cells stably-transduced with the vectors were selectively expanded medium containing the neomycin antibiotic
20 derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then analyzed by Northern analysis. The probe used to detect ribozyme transcripts also cross-hybridized with human met; tRNA sequences. Referring to Figure 56, S35/HHI RNA accumulates to significant
25 levels in MT2 and CEM cells when transduced with either the retrovirus or the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives thereof, can be readily generated to deliver the desired RNA, using techniques
30 known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors described herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

5 Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

10 Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scaringe et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ -32P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M; Herschlag and Cech, 1990 Biochemistry 29, 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM MgCl₂. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Referring to Fig. 58, - Δ G refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.

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25

30 A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α -32P] CTP as one of the four ribonucleotide triphosphates. The transcription mixture was

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treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with Isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is
5 resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (kcat/KM) conditions [Herschlag and Cech 1990 *supra*]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10
10 mM MgCl₂. The RNAs were renatured by cooling to 37°C for 10-20 min. Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

15 **Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active**

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (\geq 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe *et al.*, 1990 *supra*).

Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures 25 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with \geq 2 base-paired stem II region are catalytically active.

30 **Example 35: Synthesis of catalytically active hairpin ribozymes**

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5'
35 fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozyme •substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5' 5 end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM MgCl₂ and shown to cleave the substrate efficiently (Fig. 66).

10 The target and the ribozyme sequences shown in Fig. 62 and 65 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

15 There follows an improved trans-cleaving hairpin ribozyme in which a new helix (i.e., a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion 20 of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of atleast one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself 25 in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

30 The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo*. In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

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ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA. Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF- α) in mammalian cells. Results of these experiments are shown below.

HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

VI. Chemical Modification

Oligonucleotides with 5'-C-alkyl Group

The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-C-alkynucleotides belonging to the D-allose, 2, and L-talose, 3, sugar families are shown. The family names are derived from the known sugars D-allose and L-talose (R₁ = CH₃ in 2 and 3 in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, 29-32 and Figure 77, 58-61 respectively.

This invention relates to the use of 5'-C-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-C-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-C-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 5'-C-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 5'-C-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 5'-C-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-C-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-C-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

5 Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R₁ group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More
10 preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one
15 carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy,
20 =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons,
25 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an
30 alkyl group (as described above) covalently joined to an aryl group (as described above. Carbocyclic aryl groups are groups wherein the ring
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atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, 5 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

10 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-C-alkylnucleotides; e.g. enzymatic nucleic acids having a 5'-C-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic 15 molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-C-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 5'-C-alkyl derivatives of this invention provide enhanced stability 20 to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

25 In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided 30 below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for 35 enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is 5 provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-C-alkyl substituted nucleotides 10 that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of 15 nucleic acids using 5'-C-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-C-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; 20 Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 26-29 and 56-59). These 5'-C-alkyl substituted 25 phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-O-Isopropylidine-6-Deoxy- β -D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), CuSO₄ (120 g) and conc. H₂SO₄ (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH₄OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) 30 and cooled to 0 °C. A solution of *p*-toluenesulfonylchloride (107 g, 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H₂O (2 x 500 mL), 10% H₂SO₄ (2 x 500 mL), water (2 x 300 mL), sat. NaHCO₃ (2 x 300 mL), brine (2 x 300 mL), dried over MgSO₄ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO₂ and evaporated to dryness. The residue was suspended in chloroform (750 mL), filtered, concentrated to 100 mL and purified by flash chromatography in CHCl₃ to yield 45 g (37%) of compound 4.

Example 39: Methyl-2,3-O-Isopropylidene-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and AgNO₃ (21.25 g, 125.0 mmol) in dry DMF (300 mL) t-butyldiphenylsilyl chloride (22.2 g, 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with CHCl₃ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography in CH₂Cl₂ to yield 20.0 g (75%) of compound 5.

Example 40: Methyl-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside 5 (13.5 g, 30.6 mmol) was dissolved in CF₃COOH:dioxane:H₂O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH₄OH (140 mL) and extracted with CH₂Cl₂ (500 mL). The organic layer was separated, washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), dried over MgSO₄ and evaporated to dryness. The product 6 was purified by flash chromatography using a 0-10% MeOH gradient in CH₂Cl₂. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-O-Benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranosid (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH₂Cl₂ (300 mL), washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. The product was purified by flash chromatography in CH₂Cl₂ to yield 9.5 g (89%) of compound 7.

Example 42: 1-O-Acetyl-2,3-di-O-benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac₂O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was cooled 0 °C. 98% H₂SO₄ (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. NaHCO₃ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over MgSO₄, evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH₂Cl₂ and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄, and evaporated to dryness. The product 9 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 5.7 g (80%).

Example 44: N⁴-Benzoyl-1-(2',3'-Di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)Cytosine (10).

N⁴-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 1.8 g (55%) of compound 10.

15 Example 45: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (11).

N⁶-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 11 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 2.7 g (60%).

30 Example 46: N²-Isobutyryl-9-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)guanine (12).

N²-Isobutyrylguanine (1.47 g, 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

solution of of acetates 8 (3.4 g, 5.3 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL),
5 brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 12 was purified by flash chromatography using a gradient of 0-2% MeOH in CH₂Cl₂. Yield: 2.1g (54%).

Example 47: N⁶-Benzoyl-9-(2',3'-di-O-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).

10 Nucleoside 11 (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ to yield 1.0 g (85%) of compound 15.

15 **Example 48: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)-adenine (19).**

Nucleoside 15 (0.55 g, 0.92 mmol) was dissolved in dry CH₂Cl₂ (50 mL). AgNO₃ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was
20 stirred for 2h, diluted with CH₂Cl₂ (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 0.8 g (97%) of compound 19.

25 **Example 49: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)adenine (23).**

Nucleoside 19 (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr⁺ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then
30 evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH₂Cl₂ yielded 1.1 g (80%) of 23.

Example 50: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-2'-O-t-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (27).

Nucleoside 23 (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and AgNO₃ (0.4 g, 2.3 mmol) were added. After 5 the AgNO₃ dissolved (1.5 h), t-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL), filtered into sat. NaHCO₃ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The product 27 was 10 purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-2'-O-t-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine-3'-(2-Cyanoethyl N,N-diisopropyl-phosphoramidite) (31).

15 Standard phosphorylation of 27 according to Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidite 31 in 73% yield.

Example 52: Methyl-5-O-p-Nitrobenzoyl-2,3-O-Isopropylidene-6-deoxy-β-L-Tallofuranoside (5)

20 Methylfuranoside 4 (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), p-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue 25 was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound 33. Subsequent debenzylation (NaOMe/MeOH) and silylation (see preparation of 5) led to L-talofuranoside 34 which was converted to phosphoramidites 58-61 using 30 the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers 29-32.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

5 The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried out at 37°C in the presence of 10 mM MgCl₂ as described above.

Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6,
10 A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (HH-O1 to HH-05). *HH-O 1,2,4 and 5* showed almost wild type activity (Figure 79). However, *HH-03* demonstrated low catalytic activity. Ribozymes *HH-01, 2, 3, 4 and 5* are also extremely resistant to degradation by human serum nucleases.

15 Oligonucleotides with 2'-Deoxy-2'-Alkylnucleotide

This invention uses 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic
20 nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in
25 a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides.
30 Contrary to the findings of De Mesmaeker *et al.* applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair
35

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing 5 molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which 10 are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the 15 invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not 20 include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides 25 (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other 30 related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall 35

activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 55, data not shown). The order of most aggressive nuclease activity was fetal bovine

serum, > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table 45). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in 5 overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. 10 However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound 37 may be used as a general intermediate to prepare derivatized 2'C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

15 The following are non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkylnucleotides & Other 2'-Modified Nucleotides

20 The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling 25 groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein et al. *International Publication No. WO 92/07065*; and 5 Kois et al. *Nucleosides & Nucleotides* 1993, 12, 1093-1109. The average stepwise 30 coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense.

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

15 Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 56: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylamino-pyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance
5 of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

10 **Example 57: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)**

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide
15 (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

20 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with
25 chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched
30 with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 59: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. *N,N*-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture 10 was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-*N*⁴-Acetyl-Cytidine (11)

15 Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was 20 added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The 25 resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-5 Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. *N,N*-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyi)-uridine 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

25 Example 64: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexane s as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine

15 2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

25 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

Example 68: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and 5 purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture 15 was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

20 Example 70: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved 25 in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in 30 NaHCO₃ (5 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The residue was dried over Na₂SO₄ concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The 35 residue was dried over Na₂SO₄ concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

organic extracts were dried over Na_2SO_4 , concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylsiloxy-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

5 **Example 71: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribo-furanosyl)-4-N-Acetyl-Cytosine 21**

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylsiloxy-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The 10 residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH_2Cl_2 . 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added 15 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO_4 , concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to 20 yield 21 (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribo-furanosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite) (22)

25 1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-4-N-acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction 30 mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH_2Cl_2 :MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et_3N (6.9 mL, 50 mmol) was added to a solution of POCl_3 (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C.

5 A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)uridine 23 ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% NaHCO_3 (1 x 100 mL). The

10 organic extracts were dried over Na_2SO_4 concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat.

15 NaHCO_3 (5mL). The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 x 100 mL) and washed with 5% NaHCO_3 (1 x 100 mL). The organic extracts were dried over Na_2SO_4 , concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

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Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl- β -D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH_2Cl_2 . 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO_4 , concentrated *in*

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vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **25** (1.2 g, 1.9 mmol, 68%).

Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (26)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine **25** (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product **26**, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine (28)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (Brown,J.; Christodolou, C.; Jones,S.; Modak,A.; Reese,C.; Sibanda,S.; Ubasawa A. *J. Chem .Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine **28** (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine

28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl.

5 The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraiso-
10 propylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine **29** (3.86 g, 5.8 mmol, 79%).

Example 78: 2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-O-(tetraiso-propylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL)

15 was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

20 **Example 79: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine (29)**

2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m.

25 The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield **29** (0.81 g, 1.1 mmol, 62%).

Example 80: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-t-butylbenzoyl)-adenine **29** dissolved in dry CH₂Cl₂ (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was 5 evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

10 **Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine**

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium 15 chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N- 20 (4-t-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted 30 with 20% MeOH in CH₂Cl₂.

Example 83: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The 5 organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (32)

10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuransyl)-6-N-(4-t-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the 15 dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in 20 hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidine-3',5'-O-(Tetraisopropylsiloxane-1,3-diyl)-Uridine (33)

25 Methyl(triphenylphosphoranylidine)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-O-(tetraisopropyl siloxane-1,3-diyl)-uridine 14 in CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and 30 brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropylsiloxane-1,3-diyl)-uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (34)

Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropylsiloxane-1,3-diyl)-uridine 33 (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidine-uridine 34 (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 87: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (35)

10 2'-Deoxy-2'-methoxycarbonylmethylidine-uridine 34 (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 35 (2.03 g, 3.46 mmol, 86%).

20 Example 88: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidine-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine 35 (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 36 (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidine-3',5'-O-(Tetraisopropylid-siloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropylid-siloxane-1,3-diyl)-uridine 33 (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidine-3',5'-O-(tetraisopropylid-siloxane-1,3-diyl)-uridine 37 (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

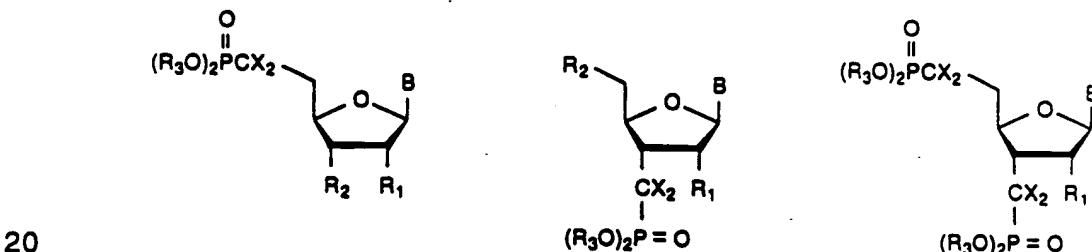
This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF₂-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman *et al.*, PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'- and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

The synthesis of 1-O-Ac-2,3-di-O-Bz-D-ribofuranose 5-dihalomethylphosphonate in three steps from 1-O-methyl-2,3-O-isopropylidene- β -D-ribofuranose 5-deoxy-5-dihalomethylphosphonate is described (e.g., for the difluoro, in Figure 87). Condensation of this suitably derivatized sugar with silylated pyrimidines and purines affords novel nucleoside 5'-deoxy-5'-dihalomethylphosphonates. These intermediates may be incorporated into catalytic or antisense nucleic acids by either chemical (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into suitably protected phosphoramidites 12a or solid supports 12b, e.g., Figure 88) or enzymatic means (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into their triphosphates, e.g., 14 Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-dihalonucleotides and nucleic acids containing such 5' and/or 3'-dihalonucleotides. The general structure of such molecules is shown below.



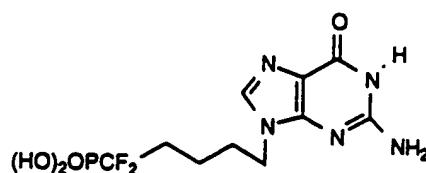
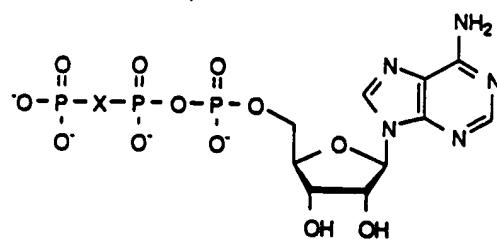
20

where R_1 is H, OH, or R, where R is a hydroxyl protecting group, e.g., acyl, alkysilyl, or carbonate; each R_2 is separately H, OH, or R; each R_3 is separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B is any nucleotide base.

The invention in particular features nucleic acid molecules having such modified nucleotides and enzymatic activity. In a related aspect the invention features a method for synthesis of such nucleoside 5'-deoxy-5'-dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

Phosphonic acids may exhibit important biological properties because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77, 349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-917) indicate that based on electronic and steric considerations α -fluoro and α,β -difluoromethylphosphonates might mimic phosphate esters better than the corresponding phosphonates. Analogues of pyro- and triphosphates 1, where the bridging oxygen atoms are replaced by a difluoromethylene group, have been employed as substrates in enzymatic processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167; Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-phosphonopentyl)guanine (2) has been utilized as a multisubstrate analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J. Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32, 9125-9128), but can still form stable complexes with complementary sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433) found that a single 3'-methyleneephosphonate linkage had a minor influence on the conformation of a DNA octamer double helix.



3

One common synthetic approach to α,α -difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (3) (Obayashi *et al.*, 5 *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.* (Martin *et al.*, 10 *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α -difluoroalkylphosphonates from primary sugar triflates using 3 was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.

15 The following are non-limiting examples showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as
5 described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

10 **Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-
difluoromethylphosphonates**

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α -difluoromethylphosphonate (4) (Martin et al., *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key
15 intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-O-isopropylidene- β -D-ribofuranose α,α -difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin et al. (*Tetrahedron Lett.* 1992, 33, 20 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I₂-MeOH, reflux, 18 h (Szarek et al., *Tetrahedron Lett.* 1986, 27, 3827) or Dowex 50 WX8 (H⁺), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-25 di-O-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak et al., *Synthesis*, 1993, 790-792) (Ac₂O, AcOH, H₂SO₄, EtOAc, 0°C. The desired 1-O-acetyl-2,3-di-O-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation 30 of silylated uracil and N⁴-acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of F₃CSO₂OSi(CH₃)₃ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 35 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, et al., *Tetrahedron*

Lett. 1987, 28, 3623-3626 and references cited therein) (SnCl_4 as a catalyst, boiling acetonitrile) to yield β -nucleosides (62% 6a, 75% 6b). Glycosylation of silylated N^6 -benzoyladenine under the same conditions yielded a mixture of N-9 isomer 6c and N-7 isomer 7 in 34% and 15% 5 yield, respectively. The above nucleotides were successfully deprotected using trimethylsilyl bromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates 8 were finally 10 purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% 8a; 87% 8b; 82% 8c).

Selected analytical data: ^{31}P -NMR (^{31}P) and ^1H -NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was CDCl_3 unless otherwise noted. 5: ^1H 15 δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, $\alpha\text{H}1$), 6.42 (s, $\beta\text{H}1$), 5.74 (d, $J_{2,3}$ 4.9, $\beta\text{H}2$), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, $\beta\text{H}3$), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, $\alpha\text{H}3$), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, $\alpha\text{H}2$), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{\text{P},\text{F}}$ 105.2), 7.67 (t, $J_{\text{P},\text{F}}$ 106.5). 6a: ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, 20 Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',\text{F}}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{\text{H},\text{F}}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{\text{P},\text{F}}$ 104.0). 8c: ^{31}P (vs DSS) (D_2O) δ 5.71 (t, $J_{\text{P},\text{F}}$ 87.9).

25 Compound 7 was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91: Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

30 The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end 35 (Figure 88 and Janda *et al.*, *Science* 1989, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

5 Example 92: Synthesis of Modified Triphosphate

The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit; John Wiley & Sons New York 1980, 10 pp. 211-218.

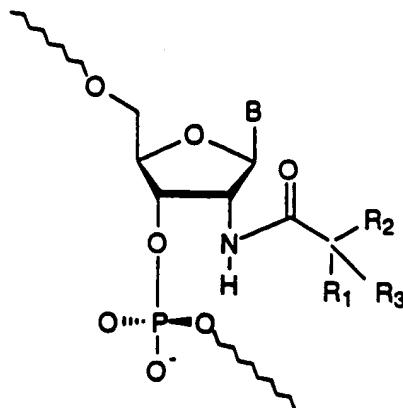
Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

The nucleoside dihalophosphonates described herein are 15 advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure. 20 These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' 25 portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:

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FORMULA I

The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In
 5 addition, either R_1 or R_2 is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, e.g., R_3NR_4 where each R_3 and R_4 independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, i.e., an amide), an alkyl group, or
 10 an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R_1 , R_2 and R_3 is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and
 15 interfere with standard base pairing interactions. Such interference will allow the formation of a compl x nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly 5 generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula I, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide 10 having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see 15 Sproat, *supra*).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol 20 [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutoxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and 25 the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ¹H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. 30 Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ¹H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using 35 standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

5 A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucleotides (e.g., adenosine, cytidine, 10 guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7 15 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ -32P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace 20 amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the 25 ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of 30 time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacylation of 3'-ends of RNA

I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

15 Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq.
20 NaHCO₃ and dichloromethane, organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 %
25 yield).

B) Preparation of the solid support and its derivatization with amino acids

Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH₂ end group) was prepared according to Haralambidis et al., Tetrahedron Lett. 1987, 28, 5199, (P denotes aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, *supra*) creating a bas -labile ester bond between amino acids

and th support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

- I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.
- II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

- 15 Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.
- 25 This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as reverting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-type .

5 In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the
10 RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).

15 A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or
20 cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 493-496.

25 Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-stranded DNA, which is an established technique for binding poly-pyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. 114, 5934-5944 (1992). Knorre, D.G., Valentin, V.V.,
30 Valentina, F.Z., Lebedev, A.V. & Federova, O.S. Design and targeted reactions of oligonucleotide derivatives 1-366 (CRC Press, Novosibirsk,
35 1992).

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin
5 gene *in vitro*. Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U.S.A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existing mutant RNA, instead of the traditional approach of inhibiting that RNA by
10 antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see
15 Figure 102), by appropriate positioning of an enzyme (or ribozyme) conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is a naturally occurring event in mammalian cells in
20 which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M., Single, F., Kohler, M., Sommer, B., and Seuberg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by
25 a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct
30 from techniques in which an active chemical group (e.g., an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is read *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*, transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDS RNA, and Alzheimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necessary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (i.e., non-human gene) to a wild type (i.e., no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase *in vivo* to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, e.g., the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. *Science* 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors.

There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion can be used when the mutant RNA creates a dominant gain of function protein (e.g., in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification (C → U and A → G). The mechanism of RNA editing in the mammalian system is postulated to be that C→U conversion is catalyzed by cytidine deaminase. The mechanism 5 of conversion of A→G has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) *Cell* 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This 10 stem-loop structure is a substrate for a nuclear double strand-specific adenosine deaminase enzyme. The deamination will result in the conversion of A→I. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

15 The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

20 Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter:

An endogenous activity in most mammalian cells and *Xenopus* oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that 25 covalently modifies its double-stranded RNA substrate. *Cell*, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This 30 stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, 35 converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

The reverted mRNA was then translated in a cell lysate and assayed
5 for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the
10 dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

While other A's in the targeted region may have been converted to I's
15 and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

20 The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

CCCGCGGTAGATCTTCTGGAGGCTTACAGTTTCTACAAACCTCC
25 CTTCAA (Seq. ID No. 1)

Referring to Figure 104, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hind* III and *Not I* sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of
30 luciferase was deleted. The sequences of the insert from the *Hind* III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAAGGGAGGTTGTGGAAAA
CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a Sac II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

5 *Xenopus* nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55, 1089-1098 (1988).

10 The target mRNA at 500ng/ μ l was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7 μ l of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin 15 and 12.5mM Tris pH 8 to a total volume of 12 μ l. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5 μ l of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies, 20 Gaithersberg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15 μ l of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96-well luminometer, and the results are 25 displayed in the graph in figure 102.

Example 98: Base changing activities

The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, 30 O.S. *Design and targeted reactions of oligonucleotide derivatives 1-366* (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* 114, 5934-5944 35 (1992). Reactive groups such as alkylators that can modify nucleotide bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have been conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these 5 conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations, 10 as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of 15 C to be read at T(Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)
- 20 2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., 25 Boston, 1987, PP.226-230.)
- 30 3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).
4. Methylation of cytosine to 5-methylcytosine
- 35 5. Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).

6. Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978) which can be done with the mutagen ethyl methane sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.

5
7. Amination of uracil to cytosine (as performed by the cellular enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

10 The following are examples of useful chemical modifications that can be utilized in the present invention. There are a few preferred straightforward chemical modifications that can change one base to another base. Appropriate mutagenic chemicals are placed on the targetting oligonucleotide, e.g., nitrous acid, or a suitable protein with such activity. Such chemicals and proteins can be attached by standard 15 procedures. These include molecules which introduce fundamental chemical changes, that would be useful independent of the particular technical approach. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 42-48.

20 The following matrix shows that the chemical modifications noted can cause transversion reversions (pyrimidine to pyrimidine, or purine to purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine to pyrimidine) are not preferred because these are more difficult chemical transformations. The footnotes refer to the specific desired chemical 25 transformations. The bold footnotes refer to the reaction on the opposite DNA strand. For example, if one desires to change an A to a G, this can be accomplished at the DNA level by using reaction #5 to change a T to a C in the opposing strand. In this example an A/T base pair goes to A/C, then when the DNA is replicated, or mismatch repair occurs this can become G/C, thus the original A has been converted to a G.

30

ISR matrix**Reverted Base**

Mutant base	A	T(U)	C	G
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A	-	Transversion	Transversion	DNA ^{5,3} /RNA ³
T(U)	Transversion	-	DNA ⁵ /RNA ⁷	Transversion
C	Transversion	RNA ² /DNA ⁶	-	Transversion
G	DNA ⁶ /RNA ⁶	Transversion	Transversion	-

- 1 Deamination of 5-methylcytosine to create thymidine.
- 2 Deamination of cytosine to create uracil.
- 3 Deamination of Adenine to be read like G (Inosine).
- 5 4 Methylation of cytosine to 5-methylcytosine.
- 5 6 Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).
- 10 7. Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).
7. Amination of uracil to cytosine. Bass *supra*. fig. 6c.

In Vitro Selection Strategy

Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity. An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction of DNAs with the appropriate base change. The cycle could then be repeated many times.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent).

Following incubation, nucleic acids are converted into complimentary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector.

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

5 Base modifying enzymatic nucleic acids (identified via in vitro selection) can be used to cause the chemical modification *in vivo*.

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing activity.

10 Such ribozymes can be used to cause the above chemical modifications *in vivo*. The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

15 Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, i.e., an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that the RNA
20 portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

25 Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see e.g., Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat,B. European Patent Application 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that site of action. While it is preferred that the R-loop structure be stable under

those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first

5 nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320). Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter or leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.

10 In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.

15 In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, e.g., an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.

20 In preferred embodiments, the first nucleic acid is a plasmid, e.g., one without a promoter or a transcription termination signal; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic acid is covalently bonded with a ligand such as a nucleic acid, protein, peptide,

25 lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group; the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, e.g., it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex;

30 no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, e.g., it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

5 R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression 10 plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21, 1019; and Meyer et al., 1986 J. Ult. Mol. 15 Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation 20 of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA heteroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into 25 a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the β -galactosidase gene. The R-loop was initiated either in the promoter region or in the 30 leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80 35 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, *supra*).

5 One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once 10 an RNA polymerase binds to the plasmid and initiates transcription, the process will continue until a termination signal is reached, or the plasmid is degraded.

This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be 15 generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (see figure 107) as described by Draper *supra*.

Ligand Targeting

Another salient feature of this invention is that the RNA used to 20 generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, etc.). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the 25 DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (see figure 108). This amino group can be directly derivatized with the ligand, such as folate (Lee 30 and Low, 1994 *J. Biol. Chem.* 269, 3198-3204). The RNA containing a 6 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent 35 any undesirable side reactions.

The RNA can also be derivatized with a heterobifunctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a

5 peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus
10 increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 *Cell* 37, 801-813; Kalderon et al., 1984 *Cell* 39, 499-509; Goldfarb et al., 1986 *Nature* 322, 641-644) and/or proteins like the transferrin (Curiel et al., 1991
15 *Proc. Natl. Acad. Sci. USA* 88, 8850-8854; Wagner et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 6099-6103; Giulio et al., 1994 *Cell. Signal.* 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide
20 or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 *Anal. Biochem* 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be
25 delivered to an appropriate intracellular cite by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 *Cancer Res.* 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex in to desired cells can also be readily accomplished.

30 In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using
35 receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

30 Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNaseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site. Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2).

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site. Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1
known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position	Target Sequences	nt. Position	Target Sequences
11	CCCCAGU C GACGCTUG	386	ACCGUGU A CUUAGACU
23	CUGAGCU C CUCUCCU	394	CUUAGACU C CAGAACG
26	AGCUCU C UGGUACU	420	CCCCCU C CCCUCUU
31	CUCUCCU A CUCAGAG	425	CUUCCCU C UGGGAG
34	UGGUACU C AGAGUUG	427	CCCCUCU U GGCAAGCC
40	UCAGAGU U GCAACC	450	AGAACCU U ACCCUAC
48	GCAACC U AGCCUCG	451	GAACCUU A CCCUACG
54	UCAGGCC U GCTAUUGC	456	UUACCCU A CGCUGCC
58	CCUOGCU A UGGCUCC	495	CCAACCU C ACCGUGG
64	UAUGGCU C CCAGCAG	510	UGCUGCU C CGUGGGG
96	CCGCACU C CUGGUCC	564	CUGAGGU C ACCGACCA
102	UCCUGGU C CUGCUAG	592	GAGAGAU C ACCAUGG
108	UCCUGCU C GGGGCU	607	AGCCAAU U UCUCGUG
115	CGGGGCU C UGUUCCC	608	GCCAAUU U CUCGUGC
119	GCUCUGU U CCCAGGA	609	CCAALUU C UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUUCU C GUCCCCC
146	CAGACAU C UGUGUCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCUCA	657	AGCUGGU U GAGAAC
158	UCCUCU C AAAAGUC	668	AACACCU C GGGCCCC
165	CAAAAGU C AUCCUGC	677	GCCCCU A CCAGCUC
168	AAGUCAU C CUGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCU C CGUGCUG	692	CAGACCU U UGUCCUG
209	AGCACCU C CUGUGAC	693	AGACCUU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUUGU C CUGCCAG
230	AAGUUGU U GGGCAAA	709	AGCCACU C CCCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAAGCC
248	ACCCCGU U GCUAAA	723	AACUUGU C AGCCCCC
253	GUUGCCU A AAAAGGA	735	CCCCGGU C CUGAGGG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CUGCCUG	763	CCGUGGU C UGUUCCC
293	AAGGAGU A UGAACUG	769	GGUCUGU U CCCUGGA
319	AGAAGAU A GCAAACC	770	GUUCGUU C CCTUGGAC
335	AUGUGCU A UUCAAAC	785	GGGUGU U CCCAGUC
337	GUGCUAU U CAAACUG	786	GGGUGUU C CCAGUCU
338	UGCUAAU C AAACUGC	792	UCCCAGU C UCGGAGG
359	GGGCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAAACCU	807	CCCAGGU C CACCUUGG
374	AAAACCU U CCUCACC	833	CAGAGGU U GAAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUUAUG
378	CCUUCCU C ACCGUGU	851	GUACACCU A UGGCAAC

863	AACGACT	C CUCUCUG	1408	UCCAGAU	C UUGAGGG
866	GACJCCU	U CUCGGCC	1410	GAGAUCU	U GAGGGCA
867	ACUCCUU	C UCAGCCA	1421	GGCACCU	A CCUCUGU
869	UCCUUCU	C GGCGAG	1425	CCUACCU	C UGUCCGG
881	AAGGCCU	C AGUCAGU	1429	CCUCUGU	C GGGCCAG
885	CCUCAGU	C AGUGUGA	1444	GAGCACU	C AAAGGGGA
933	GUGGAGU	A AUACUGG	1455	GGGAGGU	C ACGCGCG
936	CAUAAU	A CGGGGGA	1482	AUGUGCU	C UCCCCCCC
978	UGACCAU	C UACAGCU	1484	GUGCUCU	C CCCCCCGG
980	ACCAUCU	A CAGCUUU	1493	CCCGGGU	A UGAGAUU
986	UACAGCU	U UCGGGCG	1500	AUGAGAU	U GUCAUCA
987	ACAGCTU	U CGGGCGC	1503	AGAUUGU	C AUCAUCA
988	CAGCUUU	C CGGGGCC	1506	UUGUCAU	C AUCACUG
1005	ACGUGAU	U CUGACGA	1509	UCAUCAU	C ACUGUGG
1006	CGUGAUU	C UGACGAA	1518	CUGUGGU	A GCAGCG
1023	CAGAGGU	C UCAGAA	1530	CCGCAGU	C AUAUAGG
1025	GAGGUCU	C AGAAGGG	1533	CAGUCAU	A AUGGGCA
1066	CCACCU	A GAGCCAA	1551	CAGGCCU	C AGCACGU
1092	AUGGGGU	U CCAGCCC	1559	AGCACGU	A CCUCUAU
1093	UGGGGUU	C CAGGCCA	1563	CGUACCU	C UAUUACC
1125	CCCAGCU	C CUGCUGA	1565	UACCUUC	A UAACCGC
1163	CGCAGCU	U CUCCUGC	1567	CCUCUAU	A ACCGCCA
1164	GCAGCUU	C UCCUGCU	1584	GGAAGAU	C AAGAAAU
1166	AGCUUCU	C CUGCUU	1592	AAGAAAU	A CAGACUA
1172	UCCUGCU	C UGCAACC	1599	ACAGACU	A CAACAGG
1200	GCCAGCU	U AUACACA	1651	CAGGCCU	C CCUGAAC
1201	CCAGCUU	A UACACAA	1661	UGAACCU	A UCCCGGG
1203	AGCUUAU	A CACAAGA	1663	AACTUAU	C CCGGGAC
1227	GGGAGCU	U CGUGUCC	1678	AGGGCCU	C UUCCUCG
1228	GGAGCUU	C GUGUCCU	1680	GGCUCU	U CCUCGGC
1233	UUCGUGU	C CUGUAUG	1681	GCUCUUU	C CUCGGCC
1238	GUCCUGU	A UGGCCCC	1684	UCUUCU	C GGCCUUC
1264	GAGGGAU	U GUOOGGG	1690	UCGGCCU	U CCCAUAU
1267	GGAUUGU	C CGGGAAA	1691	CGGCCUU	C CCAUAUU
1294	AGAAAAAU	U CCCAGCA	1696	UUCCCAU	A UGGUGGG
1295	GAARAUU	C CCAGCAG	1698	CCCAUAU	U GGUGGCA
1306	GCAGACU	C CAAUGUG	1737	AAGACAU	A UGCCAUG
1321	CCAGGCU	U GGGGGAA	1750	UGCAGCU	A CACCUAC
1334	AAACCAU	U GCGCGAG	1756	UACACCU	A CCGGCCC
1344	CCGAGCU	C AAGUGUC	1787	AGGGCAU	U GUCCUCA
1351	CAAGUGU	C UAAAGGA	1790	GCAUUGU	C CUCAGUC
1353	AGUGUCU	A AAGGAUG	1793	UUGUCCU	C AGUCAGA
1366	UGGCACU	U UCCCACU	1797	CCUCAGU	C AGAUACA
1367	GGCACUU	U CCCACUG	1802	GUAGAU	A CAACAGC
1368	GCACUUU	C CCACUGC	1812	ACAGCAU	U UGGGGCC
1380	UGCCCAU	C GGGGAAU	1813	CAGCAU	U GGGGCCA
1388	GGGGPAU	C AGUGACU	1825	CCAUGGU	A CCUGCAC
1398	UGACUGU	C ACUCGAG	1837	CACACCU	A AAACACU
1402	UGUCACU	C GAGAUU	1845	AAACACU	A GCCCACG

1856	CACGCAU C UGAUCUG	2189	UAUUUAU U GAGUGUC
1861	AUCUGAU C UGGAGUC	2196	UGAGUGU C UUUUAUG
1865	GAUCUGU A GUCAUCAU	2198	AGUGUCU U UUAUGUA
1868	CUGUAGU C ACAUGAC	2199	GUGUCUU U UAUUGAG
1877	CAUGACU A AGCCAAG	2200	UGUCUUU U AUGUAGG
1901	CAAGACU C AAGACAU	2201	GUCCUUU A CGTJAGGC
1912	ACAUGAU U GAUGAU	2205	UUUAUGU A GGCUAAA
1922	UGGAUGU U AAAGUCU	2210	GUAGGCCU A AAUGAAC
1923	GGAUUUU A AAUCUA	2220	UGAACAU A GGUCUCU
1928	UUAAAGU C UAGCCUG	2224	CAUAGGU C UCTUGGCC
1930	AAAGUCU A GCGCGAU	2226	UAGGUCU C UGGCCUC
1964	GAGACAU A GCCCCAC	2233	CTGGCCU C ACGGAGC
1983	AGGACAU A CAACUGG	2242	CGGAGCU C CCAGUCC
1996	GGGAAA U CUGAAC	2248	UCCCAGU C CAUGUCA
2005	UGRAACU U GCUGGCC	2254	UCCAGGU C ACAUUCA
2013	GCUGCCU A UUGGGUA	2259	GUACAU U CRAGGUC
2015	UGCCUAU U GGGUAUG	2260	UCACAUU C AAGGUCA
2020	AUUGGGU A UGCUGAG	2266	UCAAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGCCCU C CAUAGAC	2282	CAGUUGU A CRGGJUG
2061	CCUCCAU A GACAUGU	2288	UACAGGU U GUACACU
2071	CAUGUGU A GCACTAA	2291	AGGUUGU A CRUGCA
2076	GUAGCAU C AAAACAC	2321	AAAAGAU C AAAUGGG
2097	CCACACTU U CCTUGACG	2338	UGGGACU U CUCATUG
2098	CACACUU C CTGACGG	2339	GGGACUU C UCAUTGG
2115	GCCAGCU U GGGCACU	2341	GACUUCU C AUUGGCC
2128	CGCGUGU C UACUGAC	2344	UUCUCAU U GGCAAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCCCAG
2145	CAACCCU U GATGATA	2359	CGCCUU U CCCCAGA
2152	UGAUGAU A UGUAUU	2360	UGCCUUU C CCCAGAA
2156	GAUAGGU A UUUAUUC	2376	GAGUGAU U UUUCUAU
2158	UAUGUAU U UAUCAU	2377	AGUGAUU U UUCUAUC
2159	ACGUAUU U AUUCAU	2378	GUGAUUU U UCUAUCG
2160	UGUAUUU A UUCAUU	2379	UGAUUUU U CUACCGG
2162	UAUUUAU U CAUUGU	2380	GAUUUUU C UAUCCGC
2163	AUUUAU C AUUUGUU	2382	UUUUUCU A UCGGCAC
2166	UAUCAU U UGUAUU	2384	UUUCUAU C GGCACAA
2167	AUUCAU U GUUAUU	2399	AAGCACU A UAUUGAC
2170	CAUUGU U AUUUUAC	2401	GCACAUU A UGGACUG
2171	AUUUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAU U UUACCG	2417	UAADGGU U CACAGGU
2174	UGUUAU U UACCGC	2418	AAUGGUU C ACAGGUU
2175	GUUAUUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUAUUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUUACG	2433	CAGAGAU U ACCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCCAGUG
2186	AGCUAUU U AUUGAGU	2448	GAGGCCU U AUUCCUC
2187	GCTUAUU A UUGAGUG	2449	AGGCUU A UUCCUCC

2451	GCCUUUAU U CCUCUCCU	2750	UAUGUGU A GACAAGC
2452	CCUJAUU C CUCCCCU	2759	AACAGCU C UCGCUCU
2455	UAUCCU C CCUUCCC	2761	AAGCUCU C GCUCUGU
2459	CCUCCCU U CCCCCCA	2765	UCUCGCU C UGUCACC
2460	CUCUUU C CCCCCAA	2769	GCUCUGU C ACCCAGG
2479	GACACCU U UGUUAGC	2797	GUGCAAU C AUGGUUC
2480	ACACCUU U GUUAGCC	2803	UCAUJGU U CACUGCA
2483	CCUUUGU U AGCCACC	2804	CAUGGUU C ACUGCAG
2484	CUUUGUU A GGCACCU	2813	CUGCAGU C UUGACCU
2492	GCCACCU C CCCACCC	2815	GCAGUCU J GACCUUU
2504	CCCACAU A CAUUCU	2821	UUGACCU J UUGGGCU
2508	CAUACAU U UCUGCCA	2822	UGACCUU U UGGGCUC
2509	AUACAUU U CGGCCAG	2823	GACCUUU U GGGCUCA
2510	UACAUU C UGCGAGU	2829	UUGGGCU C AAUGUAU
2520	CCAGUGU U CACAAAG	2837	AAGUGAU C CUCCCAC
2521	CAUGUGU C ACAADGA	2840	UGAUCCU C CCACCU
2533	UGACACU C AGOGGUC	2847	CCCACCU C AGCTUCC
2540	CAGCGGU C AUGUCUG	2853	UCAGCCU C CUGAGUA
2545	GUCAUGU C UGGACAU	2860	CCUGAGU A GCUGGGA
2568	AGGGAAU A UGCCCCA	2872	GGACCAU A GGCUCAC
2579	CCAAGCU A UGCCUUG	2877	AUAGGCU C ACAACAC
2585	UAUGCCU U GUCCUCU	2899	GGCAAU U UGAUUUU
2588	GCCUUGU C CUCUUGU	2900	GCAAU U GAUUUUU
2591	UUGGUCCU C UUGGUCCU	2904	AUTUGAU U UUUUUUU
2593	GUCCUCU U GUCCUGU	2905	UUUGAUU U UUUUUUU
2596	CUCUUGU C CGUUUUG	2906	UUGAUU U UUUUUUU
2601	GUCCUGU U UGCAUUU	2907	UGAUUUU U UUUUUUU
2602	UCCUGUU U GCATUUC	2908	GAUUUUU U UUUUUUU
2607	UUGCAU U UCACUGG	2909	AUUUUUU U UUUUUUU
2608	UUGCAU U CACUGGG	2910	UUUUUUU U UUUUUUU
2609	UGCAUUU C ACTGGGA	2911	UUUUUUU U UUUUUUU
2620	GGGAGCU U GCACAU	2912	UUUUUUU U UUUUUUC
2626	UUGCACU A UUGCAGC	2913	UUUUUUU U UUUUUCA
2628	GCACAU U GCAGCUC	2914	UUUUUUU U UUUUCAG
2635	UGCAGCU C CAGUUUC	2915	UUUUUUU U UUUCAGA
2640	CUCCAGU U UCCUGCA	2916	UUUUUUU U UUCAGAG
2641	UCCAGUU U CCTUGCAG	2917	UUUUUUU U UCAAGAGA
2642	CCAGUUU C CUGCAGU	2918	UUUUUUU U CAGAGAC
2653	CAUGUAU C AGGGUCC	2919	UUUUUUU C AGAGACG
2659	UCAGGGU C CTGCAAG	2931	ACGGGGU C UCGAAC
2689	CCAAGGU A UJUGAGG	2933	GGGGUCU C GCAACAU
2691	AAGGUAU U GGAGGAC	2941	GCAACAU U GCCCAGA
2700	GAGGACU C CCUCCCA	2951	CCAGACU U CCUUUGU
2704	ACUCCCU C CCAGCUU	2952	CAGACTU C CUUUGUG
2711	CCCAGCU U UGGAAGG	2955	ACUUCCU U UGUGUUA
2712	CCAGCUU U GGAAGGG	2956	CUUCCU U GUGGUAG
2721	GAAGGGU C AUCCGCG	2961	UUUGJGU U AGUAAAU
2724	GGGUCAU C CGCGUGU	2962	UUGUGUU A GUUAAUA
2744	UGUGUGU A UGUGUAG	2965	UGUUAGU U AAUAAAG

2966	GUUAGUU A AUAAAGC
2969	AGUUUAU A AAGCUU
2975	UAAAAGCU U UCUCAAC
2976	AAAGCUU U CUCAACT
2977	AAGCUUU C UCAACUG
2979	GUUUUCU C AACUGCC

Table 3

Mouse ICAM HH Target Sequence

863	AuCAGAU U CcUCUgG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU U cCGGaga	1421	ccCACCU A CuUuUGU
869	UCuUccU C augCAAG	1425	aCTgCCU U gGUaGaG
881	AuGGCuU C AacCcGU	1429	uCUCUaU U GccCCUUG
885	CCUuggGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c ADuCUGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU U CGGGuGC	1482	AguUGU U UgCuCCC
978	UaACagU C UACaACU	1484	cUGuUCU U CCuCaugG
980	ACagUCU A CaACUU	1493	CuguGcU U UGAGAAC
986	UACAAcCU U UuCaGCU	1500	AUGAaAU c aUggUCC
987	ACAaCuu U uCaGCUc	1503	gGAcUzU a AUCAUuc
988	CAaCuuU U CaGGuCC	1506	UUaUguU U AUaACCg
1005	ACcaGAU c CGGgaGA	1509	cuAccAU C ACCGUGU
1006	uGAGAgU C UGggGAA	1518	ucaUGGU c ccAGgCG
1023	ugGAGGU C UCGGAAG	1530	CuaaAaU C AUuucUGG
1025	GAGGUcU C gGAAGGG	1533	ugGUCAU U gUGGGCc
1066	CCACuCU c aAaaauAA	1551	CAuGCCU U AGCAgeU
1092	AcuGGAU c uCAGgCC	1559	AGCACCU c CCCaccU
1093	UGGaccU U CAGCCaA	1563	CuUAuqU U UAUAAACC
1125	CCCAacCU C uUcUUGA	1565	UAugUuU A UAACCGC
1163	CGaAGCU U CUCuUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C UuuUGCU	1584	GaAAAGAU C AgGAuAU
1166	AGCUUCU U uUGCUCU	1592	AgGAAU A CAaguUA
1172	UCCUGuU U aaaaACC	1599	ACAaguU A CAgaAGG
1200	cuCUGCU c cUccACA	1651	CcCACCU C CCTUGAGC
1201	gCuGCCU U UgaACAg	1661	gaAAACC U UCCuuuG
1203	AcuUUU:U U CACcAGu	1663	AACCUuU C CuuuGAA
1227	GGuAcaU a CGUGUgc	1678	AGGaccU C agCCUgG
1228	GaAGCUU C uUuUgCU	1680	aGCCacU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCacUU C CUcUGcC
1238	GUgCUGU A UGGUCCU	1684	acUUCCU C ugGcUgu
1264	GAaGGgU c GUgCaaG	1690	ccGGaCU U uCgAUcU
1267	uGAgaGU C UGGGgAA	1691	CGGacUU U CgAUcUU
1294	AGcAgAU a CugAGCc	1696	UgCCCAU c ggGGUGG
1295	GAGgggU C UcAGCAG	1698	CggAUAU a ccUGGag
1306	GCAGACU C ugAaaUG	1737	gAGACCU c UaccAgc
1321	gaAGGCU c agGagGA	1750	gGCgCU c CACCUca
1334	AACCCAU c UCCuaAa	1756	gAagCCU U CCuGCC
1344	aUGAGCU C gAGaGUg	1787	gaGacAU U GUCCeCA
1351	ugAaUGU a UAAguuA	1790	GCAUUGU U CUcuaau
1793	UggGUCCU C ggCugGA	2173	UUagagU U UUACCAG
1797	CacCAGU C AcAUAA	2174	UagagUU U UACCAGC
1802	accAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUAUUG
1825	CCAcGcU A CCUcugC	2185	CAGCUAU U UAUUGAG
1837	CAugCCU U uAgCuCC	2186	AGCUAUU U AUUGAGU
1845	cgAgCCU A GGCCACC	2187	GTUAUUU A UUGAGUa

1856	CggacUu u cGAUCUu	2189	UAUUUAU U GAGUacc
1861	AcaUGAU a UccAGUA	2196	caAcUcU u cUUgAUG
1865	cAcuUGU A GcCUCAG	2198	geaGcCU c UUAUGUu
1868	CaccAGU C ACAUaAA	2199	GccUCUU a UgUuUAu
1877	CAUGccU u AGCagcu	2200	UcUuccU c AUGcAaG
1901	uAAAACU C AAGggAc	2201	aaguUUU A UGUcGGC
1912	AuAUtagU a GAUcaSU	2205	JUUAUGJ c GGCCugA
1922	UGaAUgU a uAGGua	2210	GgAGaCU c AgUGgcu
1923	UGAUUGU c AgGUaUc	2220	cuGcAU u GUCCUCJ
1928	UUAgAGU u UuaCCaG	2224	CicAGGU a UCCauCC
1930	AcAGUuU u aCCaGcU	2226	UggAUUCJ C aGGCCgC
1964	GAGACAU u GuCCCca	2233	CxGaCCJ C cuGGAGg
1983	AGGAuAU A CRAgUua	2242	uGGAGCU a gCgGaCC
1996	acGGAgAU A CGAGGCC	2248	UauCcU C CAUccCA
2005	UGgAgCU a GCgGaCc	2254	UCCAAuU C ACAcUgA
2013	GCuAuuU A UUGaGUA	2259	aUCACAU U CAcGGUg
2015	UGCCcAU c GGGugG	2260	UCACAUU C AcGGUgc
2020	gSUGGUU c UUCUGAG	2266	ggAAwGU C ACCAGG
2039	gCuGgCU a gCAGAgG	2274	ACCAgAU c CuGgaGa
2040	CuGACCu c CuGgAGg	2279	GaAggGU c GUgCaAG
2057	UGCuCCJ C CAcAucC	2282	aAGcUGU u ugaGcUG
2061	CuaCCAU c acCgUGU	2288	UAuAAgU U aUggccU
2071	CAcUUGU A GCcUCCAG	2291	caGUgGU u CuCUGCU
2076	GUAGCcU C AgAgCua	2321	gAAAGAU C AcADGGG
2097	CaACuCU U CuUGAUg	2338	UGaGACU c CUgcccUG
2098	CACACTU C CccccCcG	2339	GaaACeU u UCCUUuG
2115	GCCAGCU c GGaggaU	2341	GACcUcU a ccaGccU
2128	CaGCUaU u UAUUGAG	2344	UUucgAU c uuCCAgC
2130	ccUGUuU c CUGcCcC	2358	CCcaGCU c UCagCAG
2145	CAAACuCU U cuUGAUg	2359	CUGCuUU U gaaCAGA
2152	UauUaAU u UagAgUU	2360	aaCCUUU C CuuGAA
2156	uugAUGU A UUUAUuA	2376	agGUGgU U cUUCUga
2158	gAUGUAU U UAUUaAU	2377	gGUGgUU c JUCUgag
2159	AUGUAUU U ADUaAUU	2378	agGgUUU c UCUAcuG
2160	UGUAUUU A UUaAUU	2379	UGcUUUU c uCAUaaG
2162	UAUUUAU U aAUUAg	2380	aAgUUUU a UgtUCGGC
2163	AUGUAUU U AUUaaAU	2382	aUUcUCU A UUgCcCc
2166	acUUUCAU U cucUAUU	2384	aUcCaçU a GacACAA
2167	AÜguAUU U aUUAAuU	2399	AAaCACU A UgUGGAC
2170	uAUUUaU U AaUUUAg	2401	aagCUGU u UGagCUG
2171	AgUUGUU u UgeUccC	2411	uACUGGU c AgGaUgC
2417	gAAUDGGU a CWAacGU	2691	AAuGUcU c cGAGGccC
2418	AcUGGau C ucAGGcc	2700	GAaGcCU u CCUgCCC
2425	CAugGGU c gAGgGuU	2704	gaccUCU a CCAGCcU
2426	AuuuaUU u AGAGUuU	2711	CCCAGCU c Ucagc2G
2433	uAGAGUu U uaccAGc	2712	gagGucU c GGAAGGG
2434	AGAGUuU u accAGcu	2721	GIAAGGGU C gUgCaaG
2448	GAAGCCU U ccUgCcC	2724	GGuaCAU a CGuGUGC
2449	AaGCCUU c cUgCCCC	2744	gGUgGGU c cGUgCAG

2451	GCCUguU	U CCUgCCU	2750	UAUuUaU	u GAgUAcc
2452	CCUguUU	C CUgCCUc	2759	cCggacU	u UCGaUCU
2455	gAagCCU	u CCUgCCC	2761	AgGaccU	C aCcUUGc
2459	CCaCaCU	U CCCCCC	2765	UuUuGCU	C UGeCgCu
2460	CaCaCUU	C CCCCCCg	2769	agUCUJU	C AaaCAGG
2479	GAgACCU	c UaccAGC	2797	aUGaAAU	C AUGGUcC
2480	uCACCGU	U GUgAuCC	2803	UCAUGGU	c CcagGcg
2483	CCaaUGU	c AGCCACC	2804	ggUGGgU	C cgUGCAG
2484	CUUUuUU	c accAguc	2813	CUccGGu	C cUGACCC
2492	agCACCU	C CCCACCU	2815	aCAGUCU	a aAACUUU
2504	CCCACCU	A CuUUUgU	2821	cUGACCU	c cUGGagg
2508	uAUcCAU	c caUcccA	2822	gGAgcCU	c cGGacTJu
2509	uUAgAgU	U uUaCCAG	2823	ugCCJUU	a GcUCCcA
2510	UAgAgUU	u UaCCAGC	2829	cUGGacU	a uAaUcAU
2520	CuuuUGU	U CcCAAUG	2837	AgGUGgU	u CUuCuGa
2521	CAGcaUU	u ACccUca	2840	UGAGaCU	C UggCCUg
2533	UGAugCU	C AGguaUC	2847	CCaAucU	C AGCCaCC
2540	CAGCaGU	C cgcUgUG	2853	gCAGCCU	C uUauGUu
2545	GUgcUGU	a UGGUcCU	2860	gCcaAGU	A aCUGuGA
2568	guGAGU	c UGUcAA	2872	GGACCUU	c aGCcaAg
2579	auAAGU	A UGGCcU	2877	uUccGCU	a cCAuCAC
2585	ouggCCaU	U GUuCUCU	2899	cGgAcuU	U cGAuCuU
2588	GCauUUGU	u CUCUaaU	2900	uuAAwUU	a GAgUUUU
2591	UgGUuCU	C UgeUCCU	2904	AcUUcAU	U cUcUaUU
2593	cuUcUuU	U GcuCUGC	2905	c'JUcAUU	c UcUaU
2596	CUuUUGU	u CccaaUG	2906	UUGAUgU	a UUUaUUa
2601	acCgUGU	a UuCgUUU	2907	UGuaUUU	a UUaaUUU
2602	UCCaGcU	a cCAUccc	2908	GAageUU	c UUUUgCU
2607	cUcGgAU	a UaccUOG	2909	AgcUUcU	U UUgcUcU
2608	caGCAgU	c CgCUUG	2910	UgUaUUU	a UUaaUUU
2609	gGAAlUgU	C ACCaGGA	2911	UgUaUUU	a UUaaUUU
2620	AGGAccU	c aCcUUGc	2912	UUgUUcU	c UaaUgUC
2626	UUuCgaU	c UUccAGC	2913	UUUcUcU	a cUggUCA
2628	GCACacU	U GUAGCcU	2914	UgcUUUU	c UcaUaAG
2635	UuCAGCU	C CgGUccU	2915	aUUUaUU	a aUUuAGA
2640	ggCCuGU	U UCCUGCc	2916	UaUUcgu	U UccGgGAG
2641	cccAGcU	c uCaGCAG	2917	aUUcguU	U ccGgGAGA
2642	CCuGUUU	C CUGCcuc	2918	UUcgUUU	c CgGAGAg
2653	uAcUGgU	C AGGaUgC	2919	UUcUcaU	a AGgGuCG
2659	gaAGGGU	C gUGCAAG	2931	ugGaGGU	C UCGgAAg
2689	CuAAuGU	c UccQAGG	2933	GaGGUCU	C GgAAggg
2941	GagACAU	U GuCCccA			
2951	CCAcgCU	a CCUcUGc			
2952	CAcGagU	C CgcUGUG			
2955	AgUgaCU	c UGUGUcA			
2956	uUUCCU	U GaaUcAa			
2961	UcUGUGU	c AGccAcU			
2962	uUGUaUU	u aUUAAUu			
2965	UuUgAaU	c AAUAAAG			

2966 GcUgGcU A gcAgAGG
2969 AaUcAAU A AAQcUUU
2975 UAgAGuU U UacCAgC
2976 gAgGgUU U CUCuACU
2977 AAGCUgU u UgAgsCUG
2979 uCaUUCU C uAUUGCC

Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
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11	CAGCGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	A GUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
31	CUTUGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CAACUCU CUGAUGAGGCCGAAAGGCCGAA AGUAGCA
40	AGGUUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUGC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGTUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
64	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
96	GGACCA G CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCCUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGCTG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACTUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCACG CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUCACAG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCAAC CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
230	UAUGCCC CUGAUGAGGCCGAAAGGCCGAA ACRACUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACGGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACUCCUU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCCUCU
335	GUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGCAC
338	CGAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACUGCCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

427	GGCUGCC CUGAUGAGGCCGAAAGGCCGAA AGAGGGG
450	GUAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
451	CGUAGGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
456	GGCAGCG CUGAUGAGGCCGAAAGGCCGAA AGGGUAA
495	CCACGGU CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
510	CCCCACG CUGAUGAGGCCGAAAGGCCGAA ACCAGCA
564	UGGUUCGU CUGAUGAGGCCGAAAGGCCGAA ACCUCAG
592	CCAUGGU CUGAUGAGGCCGAAAGGCCGAA AUCUCUC
607	CACGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
608	GCACGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
609	GGCACGA CUGAUGAGGCCGAAAGGCCGAA AAUUGGG
611	GCGGCAC CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
656	GUUCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUC
657	UGJUCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
668	GGGGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUGUU
677	GAGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGGC
684	AGGUCUG CUGAUGAGGCCGAAAGGCCGAA AGCUGGU
692	CAGGACA CUGAUGAGGCCGAAAGGCCGAA AGGUUCG
693	GCAGGAC CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
696	CUAGGAG CUGAUGAGGCCGAAAGGCCGAA ACAAAAGG
709	UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUCCU
720	GGCUGAC CUGAUGAGGCCGAAAGGCCGAA AGUUGUG
723	GGGGGCU CUGAUGAGGCCGAAAGGCCGAA ACAAGUU
735	CCUCUAG CUGAUGAGGCCGAAAGGCCGAA ACCGGGG
738	CCACCU CUGAUGAGGCCGAAAGGCCGAA AGGACCC
765	GGGAACA CUGAUGAGGCCGAAAGGCCGAA ACCACGG
769	UCCAGGG CUGAUGAGGCCGAAAGGCCGAA ACAGACC
770	GUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGAC
785	GACUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGCCC
786	AGACUGG CUGAUGAGGCCGAAAGGCCGAA AACAGCC
792	CCUCCGA CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
794	GGCCUCC CUGAUGAGGCCGAAAGGCCGAA AGACUGG
807	CCAGGUG CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
833	GGGGUUC CUGAUGAGGCCGAAAGGCCGAA ACCUCUG
846	CAUAGGU CUGAUGAGGCCGAAAGGCCGAA ACUGUGG
851	GUUGCCA CUGAUGAGGCCGAAAGGCCGAA AGGUGAC
863	CGAGAAC CUGAUGAGGCCGAAAGGCCGAA AGUCGUU
866	GGCOGAG CUGAUGAGGCCGAAAGGCCGAA AGGAGUC
867	UGGCGGA CUGAUGAGGCCGAAAGGCCGAA AAGGAGU
869	CUUUGGCC CUGAUGAGGCCGAAAGGCCGAA AGAAGGA
881	ACUGACU CUGAUGAGGCCGAAAGGCCGAA AGGCCUU
885	UCACACU CUGAUGAGGCCGAAAGGCCGAA ACUGAGG
933	CCAGUAU CUGAUGAGGCCGAAAGGCCGAA ACUGCAC
936	UCCCCAG CUGAUGAGGCCGAAAGGCCGAA AUUACUG
978	AGCUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGUCA
980	AAAGCUG CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
986	CGCCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUGUA
987	GCGCCGG CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
988	GGCGCCG CUGAUGAGGCCGAAAGGCCGAA AAAGCUG

1005	UCGUCAAG CUGAUGAGGCCGAAAGGCCGAA AUCACTU
1006	UUCGUCA CUGAUGAGGCCGAAAGGCCGAA AAUCACG
1023	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACCUUCG
1025	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCCJC
1066	UUGGCUC CUGAUGAGGCCGAAAGGCCGAA AGGGUUG
1092	GGGCUGG CUGAUGAGGCCGAAAGGCCGAA ACCCCAU
1093	UGGGCUG CUGAUGAGGCCGAAAGGCCGAA AACCCCCA
1125	UCAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCTUGGG
1163	GCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGCJGCG
1154	AGCAGGA CUGAUGAGGCCGAAAGGCCGAA AAGCJJC
1156	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCT
1172	GGUUGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1200	UGUGUAU CUGAUGAGGCCGAAAGGCCGAA AGCTUGGC
1201	UUGUGUA CUGAUGAGGCCGAAAGGCCGAA AAGCTUGG
1203	UCUUGUG CUGAUGAGGCCGAAAGGCCGAA AUAAAGCU
1227	GGACACG CUGAUGAGGCCGAAAGGCCGAA AGCUCCC
1228	AGGACAC CUGAUGAGGCCGAAAGGCCGAA AAGCTUCC
1233	CAUACAG CUGAUGAGGCCGAAAGGCCGAA ACACGAA
1238	GGGGCCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAC
1264	CCGGGAC CUGAUGAGGCCGAAAGGCCGAA AUCCCUC
1267	UUUCCCG CUGAUGAGGCCGAAAGGCCGAA ACAAUCC
1294	UGCCTGG CUGAUGAGGCCGAAAGGCCGAA AUUUUUCU
1295	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
1306	CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGUCUGC
1321	UUCCCCC CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
1334	CUUGGGC CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
1344	GACACUU CUGAUGAGGCCGAAAGGCCGAA AGCTUCGG
1351	UCCUUUA CUGAUGAGGCCGAAAGGCCGAA ACCTCTUG
1353	CAUCCUU CUGAUGAGGCCGAAAGGCCGAA AGACACT
1366	AGUGGGA CUGAUGAGGCCGAAAGGCCGAA AGUGCCA
1367	CAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGCC
1368	GCAGUGG CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
1380	AUUCCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
1388	AGUCACU CUGAUGAGGCCGAAAGGCCGAA AUUCCCC
1398	CUUGAGU CUGAUGAGGCCGAAAGGCCGAA ACAGUCA
1402	AGAUUCU CUGAUGAGGCCGAAAGGCCGAA AGUGACA
1408	CCCUCAA CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
1410	UGCCCCU CUGAUGAGGCCGAAAGGCCGAA AGAUUCUC
1421	ACAGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
1425	CCCGACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGG
1429	CUUGGCC CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
1444	UCCCCUU CUGAUGAGGCCGAAAGGCCGAA AGUGCUC
1455	CGGGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUCCC
1482	GGGGGGA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
1484	CCGGGGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAC
1493	AAUCUCA CUGAUGAGGCCGAAAGGCCGAA ACCGGGG
1500	UGAUGAC CUGAUGAGGCCGAAAGGCCGAA AUUCUCAU
1503	UGAUGAU CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
1506	CAGUGAU CUGAUGAGGCCGAAAGGCCGAA AUGACAA

1509 CCACAGU CUGAUGAGGCCGAAAGGCCGAA AUGAUGA
 1518 CGGCUGC CUGAUGAGGCCGAAAGGCCGAA ACCACAG
 1530 CCAUUAU CUGAUGAGGCCGAAAGGCCGAA ACUGCGG
 1533 UGCCCACU CUGAUGAGGCCGAAAGGCCGAA ADGACUG
 1551 ACGUGCU CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
 1559 AUAGAGG CUGAUGAGGCCGAAAGGCCGAA ACGUGCU
 1563 GGUUAUU CUGAUGAGGCCGAAAGGCCGAA AGGUACG
 1565 GCGGUUA CUGAUGAGGCCGAAAGGCCGAA AGAGGUA
 1567 UGGCGGU CUGAUGAGGCCGAAAGGCCGAA AUAGAGG
 1584 AUUUCUU CUGAUGAGGCCGAAAGGCCGAA AUUUCCC
 1592 UAGUCUG CUGAUGAGGCCGAAAGGCCGAA AUUUCUU
 1599 CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCUGU
 1651 GUUCAGG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
 1661 CCGGGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUCA
 1663 GUCCCOGG CUGAUGAGGCCGAAAGGCCGAA AUAGGUU
 1678 CGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGGCCCU
 1680 GCGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGGCC
 1681 GGGCGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
 1684 GAAGGCG CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
 1690 AUADGGG CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
 1691 AAUDGG CUGAUGAGGCCGAAAGGCCGAA AAGGCCG
 1696 CCACCAA CUGAUGAGGCCGAAAGGCCGAA AUGGGAA
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 1737 CAUGGCA CUGAUGAGGCCGAAAGGCCGAA ADGUCUU
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 1756 GGGCCGG CUGAUGAGGCCGAAAGGCCGAA AGGUGUA
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 1790 GACUGAG CUGAUGAGGCCGAAAGGCCGAA ACAADGC
 1793 UCUGACU CUGAUGAGGCCGAAAGGCCGAA AGGACAA
 1797 UGUAUCU CUGAUGAGGCCGAAAGGCCGAA ACTUGAGG
 1802 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA ADUGAC
 1812 GGGCCCCA CUGAUGAGGCCGAAAGGCCGAA ADGCUGU
 1813 UGGCCCC CUGAUGAGGCCGAAAGGCCGAA ADGCUG
 1825 GUGCAGG CUGAUGAGGCCGAAAGGCCGAA ACCADGG
 1837 AGUGUUU CUGAUGAGGCCGAAAGGCCGAA AGGUGUG
 1845 CGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
 1856 CAGAUCA CUGAUGAGGCCGAAAGGCCGAA ADGCUG
 1861 GACUACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGAU
 1865 ADGUGAC CUGAUGAGGCCGAAAGGCCGAA ACAGAU
 1868 GUCAUGU CUGAUGAGGCCGAAAGGCCGAA ACTACAG
 1877 CUUGGCU CUGAUGAGGCCGAAAGGCCGAA AGUCAU
 1901 ADGUCUU CUGAUGAGGCCGAAAGGCCGAA AGUCUUG
 1912 AUCCAUC CUGAUGAGGCCGAAAGGCCGAA AUCAADGU
 1922 AGACUUU CUGAUGAGGCCGAAAGGCCGAA ACACCCA
 1923 UAGACTU CUGAUGAGGCCGAAAGGCCGAA AACAUCC
 1928 CAGGCUA CUGAUGAGGCCGAAAGGCCGAA ACUUTAA
 1930 AUCAGGC CUGAUGAGGCCGAAAGGCCGAA AGACUUU
 1964 GUGGGGC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
 1983 CCAGUUG CUGAUGAGGCCGAAAGGCCGAA AUGUCCU

1996	GUUCAG CUGAUGAGGCCGAAAGGCCGAA AUUUCCC
2005	AGGCAGC CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
2013	UACCAA CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
2015	CAUACCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCC
2020	CUCAGCA CUGAUGAGGCCGAAAGGCCGAA ACCCAAU
2039	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA AGUCUGU
2040	UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
2057	GUCUADG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA
2061	ACAUGTC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
2071	UUGAUGC CUGAUGAGGCCGAAAGGCCGAA ACACAGG
2076	GGGTTUU CUGAUGAGGCCGAAAGGCCGAA AUGGURAC
2097	CGTCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGUGG
2098	CGGUCAG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
2115	AGGGCCC CUGAUGAGGCCGAAAGGCCGAA AGCTGGC
2128	GUCAUA CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
2130	GGGUUCAG CUGAUGAGGCCGAAAGGCCGAA AGACAGC
2145	UAUCATC CUGAUGAGGCCGAAAGGCCGAA AGGGUUG
2152	AAAUACA CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
2156	GAUAAA CUGAUGAGGCCGAAAGGCCGAA ACATAUC
2158	AUGAATA CUGAUGAGGCCGAAAGGCCGAA AUACAU
2159	AAUGAAU CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2160	AAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2162	ACAAUUG CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2163	AAACRAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAAUA
2166	AAUAAAC CUGAUGAGGCCGAAAGGCCGAA AUGAATA
2167	AAAUUAC CUGAUGAGGCCGAAAGGCCGAA AAUGAUA
2170	GUAAAAAU CUGAUGAGGCCGAAAGGCCGAA ACAAAUG
2171	GGUAAAA CUGAUGAGGCCGAAAGGCCGAA AACAAAU
2173	CUGGUAA CUGAUGAGGCCGAAAGGCCGAA AUAAACAA
2174	GCTGGUA CUGAUGAGGCCGAAAGGCCGAA AAUAAACA
2175	AGCTGGU CUGAUGAGGCCGAAAGGCCGAA AAUAAAC
2176	UAGCUGG CUGAUGAGGCCGAAAGGCCGAA AAAUAA
2183	CAUAAA CUGAUGAGGCCGAAAGGCCGAA AGCUGGU
2185	CUCUADA CUGAUGAGGCCGAAAGGCCGAA AUAGCUG
2186	ACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUAGCU
2187	CACUCAA CUGAUGAGGCCGAAAGGCCGAA AAUAGC
2189	GACACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2196	CAUAAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
2198	UACAUAA CUGAUGAGGCCGAAAGGCCGAA AGACACU
2199	CUACAU CUGAUGAGGCCGAAAGGCCGAA AAGACAC
2200	CCUACAU CUGAUGAGGCCGAAAGGCCGAA AAAGACA
2201	GCCUACA CUGAUGAGGCCGAAAGGCCGAA AAAAGAC
2205	UUUAGCC CUGAUGAGGCCGAAAGGCCGAA ACAUAAA
2210	GUUCAUU CUGAUGAGGCCGAAAGGCCGAA AGCCUAC
2220	AGAGACC CUGAUGAGGCCGAAAGGCCGAA AUGUUCA
2224	GGCCAGA CUGAUGAGGCCGAAAGGCCGAA ACCUUAUG
2226	GAGGCCA CUGAUGAGGCCGAAAGGCCGAA AGACCUA
2233	GUUCCGU CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
2242	GGACTGG CUGAUGAGGCCGAAAGGCCGAA AGCUCCG

2248 UGACAUUG CUGAUGAGGCCGAAAGGCCGAA ACUGGG
 2254 UGAUAUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUGGA
 2259 GACCUJUG CUGADGAGGCCGAAAGGCCGAA AUGUGAC
 2260 UGACCUU CUGAUGAGGCCGAAAGGCCGAA AAUGUGA
 2266 ACCUGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGA
 2274 ACAACUG CUGAUGAGGCCGAAAGGCCGAA ACCUGGU
 2279 CCUGUAC CUGADGAGGCCGAAAGGCCGAA ACUGUAC
 2282 CAACCUUG CUGADGAGGCCGAAAGGCCGAA ACAACTG
 2288 AGUGUAC CUGADGAGGCCGAAAGGCCGAA ACCUGUA
 2291 UGCAGUG CUGADGAGGCCGAAAGGCCGAA ACACCU
 2321 CCCAUUU CUGAUGAGGCCGAAAGGCCGAA AUCUUUU
 2338 CAAUGAG CUGADGAGGCCGAAAGGCCGAA AGUCCC
 2339 CCAAUUGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCC
 2341 GGCCAAU CUGADGAGGCCGAAAGGCCGAA AGAACUC
 2344 GUUGGCC CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
 2358 CUGGGGA CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
 2359 UCTUGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCAG
 2360 UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGGCA
 2376 AUAGAAA CUGAUGAGGCCGAAAGGCCGAA AUCACUC
 2377 GAGAGAA CUGAUGAGGCCGAAAGGCCGAA AAUCACU
 2378 CGAUAGA CUGADGAGGCCGAAAGGCCGAA AAAUCAC
 2379 CCGAUAG CUGAUGAGGCCGAAAGGCCGAA AAAAUCA
 2380 GCGGAUA CUGAUGAGGCCGAAAGGCCGAA AAAAAUC
 2382 GUGCCGA CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
 2384 UUGUGCC CUGADGAGGCCGAAAGGCCGAA AUAGAAA
 2399 GUCCAUU CUGAUGAGGCCGAAAGGCCGAA AGUGCUU
 2401 CAGUCCA CUGAUGAGGCCGAAAGGCCGAA AUAGUGC
 2411 GAACCAU CUGAUGAGGCCGAAAGGCCGAA ACCAGUC
 2417 ACCUGUG CUGAUGAGGCCGAAAGGCCGAA ACCAUUA
 2418 AACCUGU CUGADGAGGCCGAAAGGCCGAA AACCAUU
 2425 AUUCUG CUGAUGAGGCCGAAAGGCCGAA ACCUGUG
 2426 AAUCUCU CUGAUGAGGCCGAAAGGCCGAA AACCUGU
 2433 ACUGGGU CUGAUGAGGCCGAAAGGCCGAA AUUCUCUG
 2434 CACUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUCU
 2448 GAGGAU CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
 2449 GGAGGAA CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 2451 AGGGAGG CUGAUGAGGCCGAAAGGCCGAA AUAGGC
 2452 AAGGGAG CUGAUGAGGCCGAAAGGCCGAA AAUAAGG
 2455 GGGAGG CUGAUGAGGCCGAAAGGCCGAA AGGAAUJA
 2459 UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGGGAGG
 2460 UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGGAG
 2479 CCTAAC A CUGAUGAGGCCGAAAGGCCGAA AGGUGUC
 2480 GGTAAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUGU
 2483 GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
 2484 AGGUGGC CUGAUGAGGCCGAAAGGCCGAA AACAAAG
 2492 GGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGC
 2504 AGAAAUG CUGAUGAGGCCGAAAGGCCGAA AUGUGGG
 2508 UGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGUAUG
 2509 CUGGCAAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAU

2510 ACUGGCA CUGAUGAGGCCGAAAGCCCGAA AAAUGUA
 2520 CAUUGUG CUGADGAGGCCGAAAGCCCGAA ACACUGG
 2521 UCAUUGU CUGADGAGGCCGAAAGCCCGAA AACACUG
 2533 GACCGCU CUGADGAGGCCGAAAGCCCGAA AGUGUCA
 2540 CAGACAU CUGADGAGGCCGAAAGCCCGAA ACGCUG
 2545 AUGUCCA CUGADGAGGCCGAAAGCCCGAA ACUGAC
 2568 UUGGGCA CUGADGAGGCCGAAAGCCCGAA AUUCCCJ
 2579 CAAGGCA CUGADGAGGCCGAAAGCCCGAA AGCUUGG
 2585 AGAGGAC CUGAUGAGGCCGAAAGCCCGAA AGGCATA
 2588 ACAGAG CUGADGAGGCCGAAAGCCCGAA ACAGGC
 2591 AGGACAA CUGADGAGGCCGAAAGCCCGAA AGGACAA
 2593 ACAGGAC CUGADGAGGCCGAAAGCCCGAA AGAGGAC
 2596 CAAACAG CUGADGAGGCCGAAAGCCCGAA ACAGAG
 2601 AAAUGCA CUGADGAGGCCGAAAGCCCGAA ACAGGAC
 2602 GAAAUGC CUGADGAGGCCGAAAGCCCGAA AACAGGA
 2607 CCAGUGA CUGAUGAGGCCGAAAGCCCGAA AUUCAAA
 2608 CCCAGUG CUGADGAGGCCGAAAGCCCGAA AAUGCAA
 2609 UCCCCGU CUGAUGAGGCCGAAAGCCCGAA AAAUGCA
 2620 AUAGUGC CUGAUGAGGCCGAAAGCCCGAA AGCUUCC
 2626 GCUGCAA CUGAUGAGGCCGAAAGCCCGAA AGUGCAA
 2628 GAGCUGC CUGADGAGGCCGAAAGCCCGAA AUAGUGC
 2635 GAAACTUG CUGAUGAGGCCGAAAGCCCGAA AGCUGCA
 2640 UGCAGGA CUGAUGAGGCCGAAAGCCCGAA ACUGGAG
 2641 CUGCAGG CUGAUGAGGCCGAAAGCCCGAA AACUGGA
 2642 ACUGCAG CUGADGAGGCCGAAAGCCCGAA AAACUGG
 2653 GGACCCU CUGAUGAGGCCGAAAGCCCGAA AUUCUG
 2659 CUUGCAG CUGAUGAGGCCGAAAGCCCGAA ACCCUUGA
 2689 CCUCCAA CUGAUGAGGCCGAAAGCCCGAA ACCUUGG
 2691 GUCCUCC CUGAUGAGGCCGAAAGCCCGAA AUACCUU
 2700 UGGGAGG CUGAUGAGGCCGAAAGCCCGAA AGUCCUC
 2704 AAGCUGG CUGADGAGGCCGAAAGCCCGAA AGGGAGU
 2711 CCUUCCA CUGAUGAGGCCGAAAGCCCGAA AGCUUGG
 2712 CCCUUCC CUGAUGAGGCCGAAAGCCCGAA AAGCUGG
 2721 CGGGAU CUGAUGAGGCCGAAAGCCCGAA ACCUUUC
 2724 ACACCGC CUGAUGAGGCCGAAAGCCCGAA AUUCACC
 2744 CUACACA CUGAUGAGGCCGAAAGCCCGAA ACACACA
 2750 GCUUGUC CUGAUGAGGCCGAAAGCCCGAA ACACATA
 2759 AGAGCGA CUGAUGAGGCCGAAAGCCCGAA AGCUUGU
 2761 ACAGAGC CUGADGAGGCCGAAAGCCCGAA AGAGCUU
 2765 GGUGACA CUGAUGAGGCCGAAAGCCCGAA AGCGAGA
 2769 CCUGGGU CUGAUGAGGCCGAAAGCCCGAA ACAGAGC
 2797 GAACCAU CUGAUGAGGCCGAAAGCCCGAA AUUGCAC
 2803 UGCAGUG CUGADGAGGCCGAAAGCCCGAA ACCAUGA
 2804 CGCAGU CUGAUGAGGCCGAAAGCCCGAA AACCAG
 2813 AGGUCAA CUGAUGAGGCCGAAAGCCCGAA ACUGCAG
 2815 AAAGGUC CUGAUGAGGCCGAAAGCCCGAA AGACTGC
 2821 AGCCCAA CUGAUGAGGCCGAAAGCCCGAA AGGUCAA
 2822 GAGCCCA CUGAUGAGGCCGAAAGCCCGAA AAGGUCA
 2823 UGAGCCC CUGAUGAGGCCGAAAGCCCGAA AAAGGUC

2829	AUCACUU CUGAUGAGGCCGAAAGGCCGAA AGCCCAA
2837	GUGGGAG CUGAUGAGGCCGAAAGGCCGAA AUCACUU
2840	GAGGUGG CUGAUGAGGCCGAAAGGCCGAA AGGAUCA
2847	GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
2853	UACUCAG CUGAUGAGGCCGAAAGGCCGAA AGGCTGA
2860	UCCCAGC CUGAUGAGGCCGAAAGGCCGAA ACUCAGG
2872	GUGAGCC CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
2877	GUGJUGU CUGAUGAGGCCGAAAGGCCGAA AGCCUAU
2899	AAAAAUCA CUGAUGAGGCCGAAAGGCCGAA AUUUGC
2900	AAAAAAUC CUGAUGAGGCCGAAAGGCCGAA AUUUGC
2904	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AUCAAU
2905	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AUCAAA
2906	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAA
2907	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAA
2908	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUC
2909	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAAU
2910	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2911	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2912	GRAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2913	UGAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2914	CUGAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2915	UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2916	CUCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2917	UCUCUGA CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2918	GUCUCUG CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2919	CGUCUCU CUGAUGAGGCCGAAAGGCCGAA AAAAAA
2931	GUUGCGA CUGAUGAGGCCGAAAGGCCGAA ACCCCGU
2933	ADGUUGC CUGAUGAGGCCGAAAGGCCGAA AGACCCC
2941	UCUGGGC CUGAUGAGGCCGAAAGGCCGAA ADGUUGC
2951	ACAAAGG CUGADGAGGCCGAAAGGCCGAA AGUCUG
2952	CACAAAG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
2955	TAACACA CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
2956	CTAACAC CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
2961	AUUAACU CUGAUGAGGCCGAAAGGCCGAA ACACAAA
2962	TAUUAAC CUGAUGAGGCCGAAAGGCCGAA AACACAA
2965	CUUUAU CUGAUGAGGCCGAAAGGCCGAA ACTAACAC
2966	GCUUUAU CUGAUGAGGCCGAAAGGCCGAA AACUAAC
2969	AAAGCUU CUGAUGAGGCCGAAAGGCCGAA AUUAACU
2975	GUUGAGA CUGAUGAGGCCGAAAGGCCGAA AGCUUUA
2976	AGUUGAG CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
2977	CAGUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGCUU
2979	GGCAGUU CUGAUGAGGCCGAAAGGCCGAA AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence
 nt. Position Ribozyme Sequence

11	CAACGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
26	AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
31	UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CGACCCU CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
40	AGGCUCAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
48	CCAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
54	CCAUACAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
58	GGAGCTA CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
64	CUGGUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
96	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
102	CCAGCAG CUGAUGAGGCCGAAAGGCCGAA ACUGGCA
108	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
115	AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
119	UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACACUCC
120	GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
146	GGAAGCC CUGAUGAGGCCGAAAGGCCGAA AGCACUG
152	AGUGGCCU CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
165	GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
185	CUGCAAG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
209	GCCAGAG CUGAUGAGGCCGAAAGGCCGAA AAGUGGC
227	GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
230	GGAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	AGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACA
248	UUUAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
253	UCUUCCU CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
263	CAGUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCU
267	UAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCCCCU
293	CAGCTCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
319	GGCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCCUU
335	GUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAG
337	CAGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUGGAC
338	UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
359	AGCGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGCAC
367	CGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
374	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUC
375	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
386	AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	AGAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
420	CGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
425	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG

427 CACUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCTG
 450 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 451 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 456 AGUGGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUAA
 495 ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUUGGUAG
 510 CCCACCG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
 564 GGADGGA CUGAUGAGGCCGAAAGGCCGAA ACCUGAG
 592 CCCAUGU CUGAUGAGGCCGAAAGGCCGAA ACCJUUC
 607 CAUGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCJ
 608 GCAUGAG CUGAUGAGGCCGAAAGGCCGAA AUUGGC
 609 GGCAGUA CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
 611 GCGGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAAATU
 636 CAGCUCU CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
 657 UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
 668 GGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCUCG
 677 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGTC
 684 AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
 692 AAGAUCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
 693 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 696 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
 709 UGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
 720 AGCTUGAA CUGAUGAGGCCGAAAGGCCGAA AGUJUGUA
 723 CGGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAAGUU
 735 UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUCUGG
 738 CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
 765 GGAAGCG CUGAUGAGGCCGAAAGGCCGAA ACGACUG
 769 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 770 UUCCAGG CUGAUGAGGCCGAAAGGCCGAA AGCHAAA
 785 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 786 AGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGC
 792 CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 794 AGUCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCCAG
 807 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
 833 GGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGCUUUG
 846 CAACGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
 851 GCUGGUUA CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 863 CCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGGU
 866 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
 867 UCUCCGG CUGAUGAGGCCGAAAGGCCGAA AACGAAU
 869 CUUGCAU CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
 881 ACGGGUU CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
 885 UCACCUC CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
 933 CCAGAAU CUGAUGAGGCCGAAAGGCCGAA AUUAUAG
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 980 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
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 987 GAGCTUGA CUGAUGAGGCCGAAAGGCCGAA AUUGTUGU
 988 GGAGCTUG CUGAUGAGGCCGAAAGGCCGAA AAAAGUUG

1005 UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUCUGGU
 1006 UUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUCA
 1023 CjuCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 1025 CCCUJUC CUGAUGAGGCCGAAAGGCCGAA AGACCUUC
 1066 UUAAJUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUGG
 1092 GGCCJUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
 1093 UUGGCUG CUGAUGAGGCCGAAAGGCCGAA AGGUCCA
 1125 UCPAGAA CUGAUGAGGCCGAAAGGCCGAA AGUJUGGG
 1163 GCPAAAG CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
 1164 AGCAAAAG CUGAUGAGGCCGAAAGGCCGAA AAGCUUC
 1166 AGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGCU
 1172 GGJJUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
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 1201 CTGUJCA CUGAUGAGGCCGAAAGGCCGAA AAGCAGC
 1203 ACJGGUG CUGAUGAGGCCGAAAGGCCGAA AAAAAGU
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 1334 UUUTAGGA CUGAUGAGGCCGAAAGGCCGAA AUUGGGUU
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 1351 UAAACUUA CUGAUGAGGCCGAAAGGCCGAA ACAUUCA
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 1366 AGUUGUA CUGAUGAGGCCGAAAGGCCGAA ACUGUUA
 1367 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
 1368 AGAGUGG CUGAUGAGGCCGAAAGGCCGAA ACAGUAC
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 1444 UCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUC
 1455 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCC
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 1797 UUUAU
 1802 GU
 1812 CUGAUGAGGCCGAAAGGCCGAA ACUGGUG
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 1856 AAGAUUG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
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 1865 CUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGUG
 1868 UUUAU
 1877 AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AGGCAUG
 1901 GUCCCCU CUGAUGAGGCCGAAAGGCCGAA AGUUU
 1912 ACUGAUC CUGAUGAGGCCGAAAGGCCGAA ACUAU
 1932 UUACU
 1923 CUGAUGAGGCCGAAAGGCCGAA ACAU
 1928 GAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCAU
 1930 CUGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUC
 1964 AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AAAC
 1983 UGGGGAC CUGAUGAGGCCGAAAGGCCGAA AUGUC
 1983 UUACUUG CUGAUGAGGCCGAAAGGCCGAA AUAU
 1983 CUGGU

1996	GGCUCAG CUGAUGAGGCCAAAGGCCGAA AUCCUCCU
2005	GGUCCGC CUGAUGAGGCCAAAGGCCGAA AGCUCCA
2013	UACUCAA CUGAUGAGGCCAAAGGCCGAA AAAUAGC
2015	CCACCCC CUGAUGAGGCCAAAGGCCGAA AUUGGCA
2020	CUCAGAA CUGAUGAGGCCAAAGGCCGAA AACCACC
2039	CCUCUGC CUGAUGAGGCCAAAGGCCGAA AGCCAGC
2040	CCUCCAG CUGAUGAGGCCAAAGGCCGAA AGGUCAAG
2057	GGAUUGUG CUGAUGAGGCCAAAGGCCGAA AGGAGCA
2061	ACACGGU CUGAUGAGGCCAAAGGCCGAA AUUGGUAG
2071	CUGAGGC CUGAUGAGGCCAAAGGCCGAA ACAAGUG
2076	UAGCUCU CUGAUGAGGCCAAAGGCCGAA AGGUAC
2097	CAUCAAG CUGAUGAGGCCAAAGGCCGAA AGAGUUG
2098	CGGGGGG CUGAUGAGGCCAAAGGCCGAA AAGUGUG
2115	AUCCUCC CUGAUGAGGCCAAAGGCCGAA AGCUGGC
2128	CUCAAUA CUGAUGAGGCCRAAGGCCGAA AUAGCUG
2130	GAGGCAG CUGAUGAGGCCAAAGGCCGAA AAACAGG
2145	CAUCAAG CUGAUGAGGCCAAAGGCCGAA AGAGUUG
2152	AACUCUA CUGAUGAGGCCAAAGGCCGAA AUUAUA
2156	UAUAAA CUGAUGAGGCCAAAGGCCGAA ACAUCAA
2158	AUUAUA CUGAUGAGGCCAAAGGCCGAA AUACAU
2159	AAUUAU CUGAUGAGGCCAAAGGCCGAA AUUCAU
2160	AAUUAU CUGAUGAGGCCAAAGGCCGAA AAUACA
2162	CUAAAU CUGAUGAGGCCAAAGGCCGAA AUAAAUA
2163	AAUUAU CUGAUGAGGCCAAAGGCCGAA AUUACAU
2166	AAUAGAG CUGAUGAGGCCAAAGGCCGAA AUGAAGU
2167	AAUUAU CUGAUGAGGCCAAAGGCCGAA AUUACAU
2170	CUAAAU CUGAUGAGGCCAAAGGCCGAA AUAAAUA
2171	GGGAGCA CUGAUGAGGCCAAAGGCCGAA AACAAACU
2173	CUGGUAA CUGAUGAGGCCAAAGGCCGAA ACUCUAA
2174	GCUGGUA CUGAUGAGGCCAAAGGCCGAA AACUCUA
2175	AGCTGGU CUGAUGAGGCCAAAGGCCGAA AAACUCU
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2183	CAUAAA CUGAUGAGGCCAAAGGCCGAA AGCUGGU
2185	CUCAAA CUGAUGAGGCCAAAGGCCGAA AUAGCUG
2186	ACUCAAU CUGAUGAGGCCAAAGGCCGAA AUUAGCU
2187	UACUAA CUGAUGAGGCCAAAGGCCGAA AAUATGC
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2196	CAUCAAG CUGAUGAGGCCAAAGGCCGAA AGAGUUG
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2200	CTUUGCAU CUGAUGAGGCCAAAGGCCGAA AGGAAGA
2201	GGCGACA CUGAUGAGGCCAAAGGCCGAA AAAACUU
2205	UCAGGCC CUGAUGAGGCCAAAGGCCGAA ACUAAA
2210	AGCCACU CUGAUGAGGCCAAAGGCCGAA AGUCUCC
2220	AGAGAAC CUGAUGAGGCCAAAGGCCGAA AUGCCAG
2224	GGAUUGGA CUGAUGAGGCCAAAGGCCGAA ACCUGAG
2226	GGGGCCU CUGAUGAGGCCAAAGGCCGAA AGAUCCA
2233	CCUCCAG CUGAUGAGGCCAAAGGCCGAA AGGUCAAG
2242	GGUCCGC CUGAUGAGGCCAAAGGCCGAA AGCUCCA

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 2254 UCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGA
 2259 CACCGUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAU
 2260 GCACCGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGA
 2266 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACACUCC
 2274 UCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUGGU
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 2288 AGGCCAU CUGAUGAGGCCGAAAGGCCGAA ACUUAAA
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 2338 CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
 2339 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUCC
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 2344 GTUGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGAAA
 2358 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2359 UCUGUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
 2360 UUCAAAG CUGAUGAGGCCGAAAGGCCGAA AAAGGUU
 2376 UCAGAAG CUGAUGAGGCCGAAAGGCCGAA ACCACCU
 2377 CUCAGAA CUGAUGAGGCCGAAAGGCCGAA AACCAACC
 2378 CAGUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCU
 2379 CUUADGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCA
 2380 GCGACAA CUGAUGAGGCCGAAAGGCCGAA AAAACUU
 2382 GGGGCAA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
 2384 UDUGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGAU
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 2401 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
 2411 GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
 2417 ACCUAUG CUGAUGAGGCCGAAAGGCCGAA ACCAUUC
 2418 GGCGUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
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 2426 AAACUCU CUGAUGAGGCCGAAAGGCCGAA AAUUAU
 2433 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUTUA
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 2449 GGGGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
 2451 AGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGC
 2452 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
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 2459 GGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGUGG
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 2480 GGATCAC CUGAUGAGGCCGAAAGGCCGAA ACGGUGA
 2483 GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAUUGG
 2484 GACUGGU CUGAUGAGGCCGAAAGGCCGAA AAAAAAG
 2492 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
 2504 ACAAAAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
 2508 UGGGAUG CUGAUGAGGCCGAAAGGCCGAA AUGGAUA
 2509 CUUGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCUAA

2510 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUUTA
 2520 CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
 2521 UGAGGGU CUGAUGAGGCCGAAAGGCCGAA AAUGCG
 2533 GATACCU CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
 2540 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCTG
 2545 AGGACCA CUGAUGAGGCCGAAAGGCCGAA ACAGCRC
 2568 UUUGACG CUGAUGAGGCCGAAAGGCCGAA ACUUCAC
 2579 CAGGCCA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
 2585 AGAGAAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
 2588 AUUAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAUUGC
 2591 AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
 2593 GCAGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
 2596 CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACRAAAAG
 2601 AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
 2602 GGGGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCUGGA
 2607 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA ADCCGAG
 2608 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
 2609 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUC
 2620 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2626 GTGGAA CUGAUGAGGCCGAAAGGCCGAA AUCCGAAA
 2628 AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
 2635 AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
 2640 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 2641 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2642 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
 2653 GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
 2659 CTUUCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 2689 CCTUCGA CUGAUGAGGCCGAAAGGCCGAA ACAUUTAG
 2691 GGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGACAUU
 2700 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2704 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
 2711 CGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2712 CCCUUC CUGAUGAGGCCGAAAGGCCGAA AGACCUUC
 2721 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 2724 GCACACG CUGAUGAGGCCGAAAGGCCGAA AUGUACC
 2744 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
 2750 GGUACTC CUGAUGAGGCCGAAAGGCCGAA AUAAAATA
 2759 AGAUUCG A CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
 2761 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2765 AGCGGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
 2769 CCUGUUU CUGAUGAGGCCGAAAGGCCGAA ACAGACU
 2797 GGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUUCAU
 2803 CGCCUGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
 2804 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
 2813 GGGUCAG CUGAUGAGGCCGAAAGGCCGAA ACCGGAG
 2815 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
 2821 CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAG
 2822 AAAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGGUCCUC
 2823 UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AAAAGCA

2829	AUGAUUA CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
2837	UCAGAAC CUGAUGAGGCCGAAAGGCCGAA ACCACCU
2840	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
2847	GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAUJUGG
2853	AACAUAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGC
2860	UCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGC
2872	CUUGGCU CUGAUGAGGCCGAAAGGCCGAA AAGGUCC
2877	GUGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
2899	AAGADCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
2900	AAAACUC CUGAUGAGGCCGAAAGGCCGAA AAAUUA
2904	AAUTAGAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGU
2905	CAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAUGAAG
2906	UAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUCAA
2907	AAAUTAA CUGAUGAGGCCGAAAGGCCGAA AAAUTACA
2908	AGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUC
2909	AGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGCU
2910	AAAUUAA CUGAUGAGGCCGAAAGGCCGAA AAAUTACA
2911	AAAUTAA CUGAUGAGGCCGAAAGGCCGAA AAAUTACA
2912	GACAUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAA
2913	UGACCAG CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
2914	CUUUAUG CUGAUGAGGCCGAAAGGCCGAA AAAAGCA
2915	UCUAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAAU
2916	CUCCCGA CUGAUGAGGCCGAAAGGCCGAA ACGAAUA
2917	UCUCCCG CUGAUGAGGCCGAAAGGCCGAA AACGAU
2918	CUCUCCG CUGAUGAGGCCGAAAGGCCGAA AAACGAA
2919	CGACCUU CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
2931	CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
2933	CCCUUUC CUGAUGAGGCCGAAAGGCCGAA AGACCTUC
2941	UGGGGAC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
2951	GCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
2952	CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2955	UGACACA CUGAUGAGGCCGAAAGGCCGAA AGUCACU
2956	UUGAUUC CUGAUGAGGCCGAAAGGCCGAA AAGGAAA
2961	AGUGGCCU CUGAUGAGGCCGAAAGGCCGAA ACACAGA
2962	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2965	CUUUAUU CUGAUGAGGCCGAAAGGCCGAA AUUCAAA
2966	CCUCUGC CUGAUGAGGCCGAAAGGCCGAA AGCCAGC
2969	AAAACUU CUGAUGAGGCCGAAAGGCCGAA AUUGAUU
2975	GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2976	AGUAGAG CUGAUGAGGCCGAAAGGCCGAA AACCCUC
2977	CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCTU
2979	GGCAAUA CUGAUGAGGCCGAAAGGCCGAA AGAAUGA

Table 6
Human ICAM Hairpin Ribozyme/Substrate Sequences
nt.
Position

	nt. Position	Hairpin Sequence	Substrate Sequence
70		GGGGGG AGAA GCUG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CAGCA GCC CCCGGCCC
86		GGAGUCCG AGAA GGFC ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	GGCCU GGC CGCAUCUU
343		CCCACUACG AGAA GUUU ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	AAACU GGC CUGAUGGG
635		GCCUUGG AGAA GGAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CUGCG GGC CCAAGGGC
653		UGUUCUCA AGAA GGUC ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	GAGCU GUU UGAGAACAA
782		AGACUGGG AGAA GCCC ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	GGGCU GUU CCCAGUCU
920		CUGCACAC AGAA GCGG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CGGCU GAC GUGGGCAG
1301		ACAUUGGA AGAA GGUG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CAGCA GAC UCCAUGU
1373		CCCCGAUG AGAA GUAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CCACU GCC CAUCGGCG
1521		AUGACUGC AGAA CCUA ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	URGCA GGC GGAGUCAU
1594		CUGUUGUA AGAA GUAU ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	AUACA GAC UACAAACAG
2008		ACCCAAUA AGAA GCAA ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	UUCU GCC UAUUGGGU
2034		UUCUGURA AGAA GUAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CCACA GAC UUACAGAA
2125		GGUCAGUR AGAA GCGAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CUCCU GUC UACUGACC
2132		GGGUUGGG AGAA GUAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CUACU GAC CCCAACCC
2276		ACCUGUAC AGAA GUAC ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	GUACA GUU QUACAGGU
2810		AAGGUCAA AGAA GCGAG ACCAGAGAAACACAGUUGGUACAUUACCUUGUA	CUECA GUC UUGACCUU

nt. Position	Sequence	Substrate
76	GGGAUCAC AGAA GUGA ACCAGAGAACACGGUUGGUACAUUACCUUGUA	UCACC GUU GUGAUCCCC
164	UGAGGAAG AGAA GUUC ACCAGAGAACACGGUUGGUACAUUACCUUGUA	GAACU GUU CUCUCUCA
252	UCAGCUCA AGAA GCUU ACCAGAGAACACGGUUGGUACAUUACCUUGUA	AAGCU GUU UGAGCUGA
284	GCACAGGG AGAA GGUG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CAGCA GUC CGCGUGGC
318	AAGGGGAC AGAA GCAC ACCAGAGAACACGGUUGGUACAUUACCUUGUA	GUGCA GUC GUGCGCUU
447	AGAGCUGG AGAA GCGG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CGCG GAC CGAGCUCU
804	UCUCCUGG AGAA GCAU ACCAGAGAACACGGUUGGUACAUUACCUUGUA	AUGCC GAC CPAGGAGA
847	UCUACCAA AGAA GUGG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CCACU GCC UUGGGUAGA
913	AGGAUCUG AGAA GCUA ACCAGAGAACACGGUUGGUACAUUACCUUGUA	UAGCG GAC CGAGAUCCU
946	AAGUUNGUA AGAA GUUA ACCAGAGAACACGGUUGGUACAUUACCUUGUA	UAACA GUC UCAZACUU
1234	CCCAAGCA AGAA GUCU ACCAGAGAACACGGUUGGUACAUUACCUUGUA	AGAGC GAC UCCUUCGG
1275	AUUCAGA AGAA GCUU ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CAGCA GAC UCTGAAAAU
1325	UGCCUUC AGAA GGAG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CUGCA GAC GGAGGCCA
1350	CCCCGAUG AGAA GCAG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CUGCU GCC CAUCGGGG
1534	ACAUUAGA AGAA GCCA ACCAGAGAACACGGUUGGUACAUUACCUUGUA	UGGCA GCC UCTUANGU
1851	GUCCACCG AGAA GUAG ACCAGAGAACACGGUUGGUACAUUACCUUGUA	CUACA GCC CGUGGAC
1880	AGAUGUA AGAA CGGU ACCAGAGAACACGGUUGGUACAUUACCUUGUA	ACGUU GAC UUCAUUCU

Position	nt.	Hairpin Ribozyme Sequence	Substrate
5		AAACGCCA AGAA GCAG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CUCUU GCC UGCACUUU
59		GGACCGCA AGAA GCAU ACCAGGAAACACAGGUUGGGUACAUUACCUGUA	AUGCU GCC UCUGGUCC
84		GGGAUACAC AGAA GCGA ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	UCGCC GUU GUGAUCCC
295		GCACAGUG AGAA GCGU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CAGCA GAC CACUGUGC
329		AAGCCCCAG AGAA GCGU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	ACGCA GUC CUCGGCUU
433		UDCCACCA AGAA GCGC ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	GCGCU GCC UGGUGGAA
626		CAUUCUUG AGAA GUGA ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	UCACU GUU CAAGRAUG
806		UCUCCACG AGAA GCAU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	AUGCU GAC CCUGGAGA
849		UCCACUGA AGAA GUGG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CCACU GCC UCAUGUGA
915		AGGGUCUG AGAA GCCA ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	UGGCG GAC CAGACCCU
1182		ACCUCCAA AGAA GCPG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CUGCG GCC UUGGGGGU
1307		AUGUAGA AGAA GCGU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CAGCA GAC UCUUACAU
1357		UGCUUUCG AGAA GCAG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CUGCA GCC GGAAAGCA
1382		UCCCGAAA AGAA GCGG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CCGGU GCC UAUUGGGA
1058		GCCCACCA AGAA GUAG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CUNCA GCC UGGGGGC
1887		AGAAGAAA AGAA GCCU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	AGGCU GAC UUCCUCU
2012		GAGUUGGG AGAA GUGU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	ACACU GUC CCCAACUC
2303		AGACUCCA AGAA GUGG ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	CCACA GCC UGGAGUCU
2539		CCUCCAC AGAA GCUU ACCAGAGAACACAGGUUGGGUACAUUACCUGUA	AAGCU GUU GUGGGAGG

Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAAU U CACACUGA	394	GUGGGUGCU U CGGAACAG
23	GCUGACUU C CUCUCUUA	420	GCAACCCU C CCAGGCGA
26	GAACUGCU C UUCCUCUU	425	CCTGGGCU U CUGGCCACC
31	CCUCUGCU C CGGGUCCU	427	UCCCGGUU U AAAAAACCA
34	CUGAAGCU C AGATAUAC	450	AAGAACCU C AUCCUGGG
40	CUCAAGGU A CAAGCCCC	451	GGGUACUU C CCCAGGGC
48	GAGAACCU C GGCCTUGGG	456	CUCGGCUU C UGCCCACCA
54	CCCCAGCU C CGUGAGCC	495	GCCACCAU C ACUGUGUA
58	CGUGUCU U UAGCUOOC	510	GCGCUGCU C CGUGGGAA
64	CAUUGGU U CAACCGU	564	AAAAAEGU U CCPACCCAC
96	CCUCUGCU C CGGGUCCU	592	GGGAGTAU C ACCAGGGA
102	CUCCUGGU C CGGGUCCG	607	GAGCCAAU U UCUCAUGC
108	GGACUGCU U GGGGAACT	608	AGCCAAU U CCTCAUGCU
115	UCCUACCU U UGUUCCCA	609	GCRAAUU C UCAUGCJJ
119	GACACUGU C CCCAACUC	611	CAUUUUCU C AUGCUUCA
120	GUUGUGAU C CCGGGGCC	656	GUACUGU U CAGAAUG
146	CCAGACCU U GGAACUCC	657	UCACUGU C AAGAAUGU
152	ACCCGGCU C CACCUCAA	668	GAACUGCU C UUCCUCUU
158	AUUUCUUU C ACGAGUCA	677	GCACCCCU C CCGCGCA
165	UGAACAGU A CUUCCCCC	684	AGGCAGCU C CGGACUUU
168	GAAGCCUU C CUGCCUCG	692	CCAGACCU U GGAACUCC
185	GGGUGGAAU C CGUGCAGG	693	CGGACUU C GAUCUUCC
209	CAGCCCCU A ADCUGACC	696	GCCUGUUU C CTGGCCUCU
227	GACCAAGU A ACUGUGAA	709	CAGCAUUU A CCCUCAC
230	CAAGCUGU U GUUGGAGG	720	CTACAACT U UUCAGCUC
237	CUGAAGCU C GACACCCC	723	CAACUUU C AGCUCCCCA
248	GGCCCCCU A CCUUAAGGA	735	CUCUGGUU C CGGGUCGC
253	CACUGCCU C AGUGGAGG	738	UCCUGCCU C GGGGUGGA
263	GAGCCAAU U UCUCAUGC	765	ACUGUGCU U UGAGAACU
267	GAAGCCUU C CUGCCUCG	769	UCUUGUGU U CCCUGGAA
293	GAAGCUCU U CAAGCUGA	770	CUUUGGUU C CCTGGGAAG
319	CGGAGGAAU C ACAAACGA	785	AGGCUGU U UCCUGCCU
335	ACUGUGCU U UGAGAACU	786	GGCCUGUU U CCUGCCUC
337	UGUGCUAU A UGGUCCUC	792	CUCUGGUU C CGGGUCGC
338	AAGCUCUU C AAGCUGAG	794	UCCUGCCU C UGAAGCUC
359	CACCGAGU C CUUGGCUU	807	GTUCAGAU A UACCUUGGA
367	CAAUGGUU U CAACCGU	833	CCUGGGGU U GGAGACUA
374	UUACCCCU C ACCCACCU	846	CUGACAGU U AUUUAUUG
375	AGAAGCCU U CCUGCCUC	851	GTCUACCU U TAGCAGCU
378	ACCCACCU C ACAGGGUA	863	CAUUGGUU U CAACCCGU
386	CGCUGUGU U UUGGAGCU	866	CCAUGGUU C CUCUGACA

867	GACCACCU C CCCACCUA	1421	GGGUACUU C CCCCCAGGC
869	CUCUUCU C UUGCGAAG	1425	ACCCACCU C CUCUUGGU
881	AADGGCUU C AACCGUG	1429	AIAACUUGU A GCGCUCAGG
885	GACCAAGU A ACUGUGAA	1444	AGAAGGCCU C AGGAGGGAG
933	UGUGUADU C GUUCCAG	1455	GGQAGAU C ACCAGGGAA
936	GCAGAGAU U UUGUGUCA	1482	AGGGUACU U CCCCCAGG
978	UUGAGAU C UACAAACUU	1484	ACUGUCU U CCUCUUGC
980	GAGAACU A CAACUUUU	1493	CCUCCCCU U GGAGACTUA
986	CUACACU U UUCAGCUC	1500	CGUGAAAU U ADGGUCAA
987	UACACUU U UCAGCUC	1503	GAAXAAGU U CCAACCAC
988	ACAACTUU U CAGCUOOC	1506	UGGGUCAU A AUUGUUGG
1005	UUCGUGAU C GUGGGGUC	1509	GCCACCAU C ACUGUGUA
1006	GUUGGGAGU A UCACCAAG	1518	GUCCUGGU C GCGGUGUJ
1023	CGGGAGGU C UCAGNAGG	1530	ACCUUGGU C AIAAUTUGU
1025	GGAGGUUCU C AGAAGGGG	1533	CUGAUCAU U GCGGGCUU
1066	CCUACCUU U GUUCCCAA	1551	UGGGGCCU C UGCUCGUA
1092	AGAGGGGU C UCAGCAGA	1559	UGGGGAAGU C CCUGUJUA
1093	AGGGGAAU C CAGCCOCU	1563	UCCUACCU U UGUUCCCCA
1125	CCCCAACU C UUGUGUAG	1565	UUACACCU A UUACCGCC
1163	ACGACGCCU U CUUUGGU	1567	ACACCUAU U ACCGCCAG
1164	CGACGCCU C UUUUGGU	1584	AGGAAGAU C AGGAUAAA
1166	ACGCUUCU U UUGGUUCG	1592	CAGGAATAU A CAAGUUAC
1172	CUUUUGGU C UGCGGGGU	1599	UACAGAU A CAGAAGGC
1200	ADCCAATU C ACACUGAA	1651	CCCCGCCU C CCTUGAGCC
1201	UUGGGCUU C UCCACAGG	1661	CUGCACUU U GCCCUGGU
1203	GGGCUCU C CACAGGU	1663	GAACAGAU C AAUGGACA
1227	UUGGAACU C CAUGUGGU	1678	GAGAACCU C GGCCUGGG
1228	GGGGGUU C GUGAUOGU	1680	GGGUUCU C CACAGGU
1233	CUCCUGGU C CUUGGUOC	1681	GGCCUGUU U CCTUGGCC
1238	UGUGUADU A UGGGUOCU	1684	CUCCUGGU A GACCUUC
1264	GGAAAGAU C AIAACGGGU	1690	CCCCACCU A CAUACAUU
1267	GUACUGU U CAAGAAGG	1691	CGGACAU U CGAUCUUC
1294	CAGAGAU U UGUGUCA	1696	CUCCUGGU C CUUGGUOC
1295	AGAGGGGU C UCAGCAGA	1698	UCAAGAU A CCTUGGAGA
1306	AGCAGACU C UUACAU	1737	GAUACACAU U CACGGUGC
1321	AACAGAGU C UGGGGAAA	1750	GUCCAUUU A CACCUAUU
1334	GUAUUCGU U CCCAGAGC	1756	CCUCUGGU C CUUGGUCC
1344	UOGGUGGU C AGGUADCC	1787	GAGAACCU C GGCCUGGG
1351	UCAGGGGU A AGAGGACU	1790	GACACUGU C CCCAACUC
1353	UAGCAGGU C AACAAADGG	1793	AUGGUOCU C ACCUGGAC
1366	AGGGUACU U CCCCCAGG	1797	UCCUCGUU U AAAAACCA
1367	GGGUACUU C CCCACGGC	1802	GUUCAGAU A UACCUUGGA
1368	GAUGGUGU C CGCTUGCC	1812	AAACAGAU C UGGGGAAA
1380	CUGCCUAU C GGGAUUGG	1813	GGGGGUU C GUGAUCGU
1388	UGGAGACTU A ACUGGAUG	1825	GCCACCAU C ACUGUGUA
1398	CUGGCUU C ACAGGACU	1837	ACCCACCU C ACAGGGUA
1402	CUGUGGUU U GAGAACUG	1845	AGAGGACU C GGAGGGGC
1408	UUCGUGAU C UGGGGUC	1856	CCCCUAAU C UGACCUUGC
1410	CGAACAUU C GAGUGGAC	1861	CAUGUGGU A UAGGUUCC

1865	UAUCCGGU A GACACAG	2198	GAADGOCU C CGAGGUCA
1868	UCACGAGU C AUAUAAA	2199	AGACUCUU A CAUGGCAG
1877	ACAGURCU U CCCCCAGG	2200	GGGUACUU C CCCCAGGC
1901	CIAAAACU C AAGGUACA	2201	GGGCUUCU C CACAGGUC
1912	GAACAGAU C AAUGGACA	2205	UUUUGUGU C AGOCACUG
1922	ADGUAAGU U AUUGCCTA	2210	UGGAGACU A ACUGGAUG
1923	UGGACGCC C ACCUUUAG	2220	GAGAACCU C GGGCGGGG
1928	GTTCAGAU A UACUUGGA	2224	ACAUACAU U CCUACCUJ
1930	UGGAGACU A ACUGGAGG	2226	CGGGACCU C AGGCCACA
1964	AGAGATTU U GUGUCAGC	2233	UCAUGCUU C ACAGAACU
1983	GAGAACCT C GGGCGGGG	2242	ACACAGCU C UCGTJAGU
1996	UGGAAGCU C UUCAAGCJ	2248	CUCCUGGU C CGGGUCGC
2005	ADGUAAGU U AUUGCCTA	2254	AUCCAAUU C ACACUGAA
2013	CGCGUCUJ A UCGGGAGG	2259	GAUCACAU U CACGGGGC
2015	CGGCUAU C GGGAGGGU	2260	AUCACAUU C ACGGUGCU
2020	UAUUGAGU A CCCUGUAC	2266	ADCAGGAU A UACRAGU
2039	CGGAGGAGU C ACRAACGA	2274	GAGCAGGU U AACADGUA
2040	CCUGACCU C CGGGAGGU	2279	GGAAAGAU C AUACGGGU
2057	CGGGUCCU C CAAUGGU	2282	ACAGGUAU U UAUGAGU
2061	GCGUCCAU U UACACCTA	2288	CCCCCGGU C CCUGGUAG
2071	AUACUTGU A GCGUCAGG	2291	CAGGAAAU A CAAGGUAC
2076	TGUAGCCU C AGGCGCTA	2321	GGAAAGAU C AUACGGGU
2097	CCAACUCUJ U GUUGAGGU	2338	UUGGGCUU C UCCACAGG
2098	CTUGACCU C CGGGAGGU	2339	GGGAACTU C CCCCAGGC
2115	UUCCGACU A GGGUCCUG	2341	GGGCCUGU C GGUGGUCA
2128	AGUGGCTGU A CCUGUJC	2344	CGCGUUGU A GACCUUCC
2130	GCCUGGUU C CGGCCUCU	2358	CCCGUGGU C CUCCACAA
2145	CCAACUCU U GUUGAGGU	2359	CCAUCCAU C CCACAGAA
2152	UUGAGAAU C UACAACUU	2360	CUUGGUU C CCUGGAAG
2156	UGACAGGU A UUUAUUGA	2376	GAACUGCU C UUCCUCUJ
2158	UGAUGUAU U UAUUAU	2377	GACUUCU U COUAAUA
2159	GAUGUAU U AUUAUUC	2378	GCUGAUU C UUUCACGA
2160	AUGUAUOU A UUUAUUC	2379	CGUCUCU C CUCUUGCG
2162	ACAUUCU A CCUUUGGU	2380	UGAUUUCU U UCACGAGU
2163	UAUUAUOU A AUUCAGAG	2382	AUUCUUCU C ACGAGUCA
2166	UGAUGUAU U UAUUAU	2384	UAUOOGGU A GACACAAG
2167	GAUGUAU U AUUAUUC	2399	UAAAUCU A UGUGGAGG
2170	GUAUUAU U AADUCAGA	2401	UGUGCUAU A UGGUCCUC
2171	CAGUUAU U AUUGAGUA	2411	CAAUUCU C AUGCUUCA
2173	UGUGCUAU A UGGUCCUC	2417	ADCAGGAU A UACAAGU
2174	UCUCUAUU A CCCCUGCU	2418	UCAUGCUU C ACAGAACU
2175	AUUUCUUU C ACAGGUCA	2425	UUAUUAU U CAGAGUUC
2176	GAAAAGU U CCAACCAC	2426	CCUGGGGU U GGAGACUA
2183	UGACAGGU A UUUAUUGA	2433	UCAGAGGU C UGACAGUJ
2185	ACAGUUAU U UAUUGAGU	2434	CGGAGGAGU C ACAAACGA
2186	CAGUUAU U AUUGAGUA	2448	UGAACAGU A CUUCCCCC
2187	AGUUAUOU A UUGAGGUAC	2449	GAAGCCUU C CGGCCUC
2189	UUAUUAU U GAGUACCC	2451	GGCCUGGU U CCUGCCUC
2196	CGUGACAGU U AUUUAUUG	2452	GCCUGGUU C CGGCCUCU

2455	AACAUUCU A CCUUUGGU	2761	CGGACUUU C GAUCUJCC
2459	CCTUGCCU C CUCCCCAA	2765	CUUUUGCU C UGGGGCCU
2460	CCUACCUU U GUUCCCAA	2769	UUCUCUAU U ACCCCUGC
2479	UUACACCU A UUACGCC	2797	CGUGAAAU U AUGGUCAA
2480	GUCCGGGU U GUGAUCCC	2803	CUCAUGC U CACAGAAC
2483	ACCUUUGU U CCCAAGGU	2804	UCAUGCU C ACAGAACU
2484	CCUUUGGU C CCAADGUC	2813	GCUCCCAU C CGACCCU
2492	GACCAACC C CCCACCUA	2815	CGGACJUU C GAUCUJCC
2504	ACCUACAU A CAUCCUA	2821	CCUGACCU C UGGGAGGU
2508	ACAUACAU U CCUACCUU	2822	UACAACUU U UCAGCUCU
2509	CAUACAUU C CCAACCUU	2823	CAACUUTU C AGCUCCCA
2510	GUCCAUU A CACCUAU	2829	UCGGJGCU C AGGUAUCC
2520	ACCUUUGU U CCCAAGGU	2837	CACAGGGU A CUUCCCCC
2521	CCUUUGGU C CCAADGUC	2840	GCACCCCU C CCAGCCGA
2533	ACAGCAAU U ACCCCUCA	2847	UUAACCCU C ACCCACCU
2540	UGGGUGCU C AGGUAUCC	2853	UUCGAUCU U CCGACUAG
2545	AGGCAGCU C CGGACUUU	2860	UCUUGUGU U CCCUGGAA
2568	CAGAGAUU U UGUGUCAG	2872	GGGCGUGU C GGUGCUCA
2579	CCUGCACT U UGCCUGG	2877	UGGAGUCU C CCAGCACC
2585	UGCGUOGU A GACCUUC	2899	AGGCAGCU C CGGACTUUU
2588	UGGCAGCU C CCACAGCC	2900	GGCUGACU U CCUUCUCU
2591	COUCUCCU C UUGCGAAG	2904	GAACUGCU C UUCCUCUU
2593	UCUCUAUU A CCCUGCU	2905	GGCUGACU U CCUUCUCU
2596	CUCCUGGU C UGGUCGC	2906	GUUGAGGU A UUUUUAAA
2601	UGGCGUAU A UGGGUCCUC	2907	CGUCUCUU C CUCUUGCG
2602	GUCCUGGU C GCGGUGU	2908	UGAUGUAU U UAUUAAA
2607	GUGGGAGU A UCACCAAG	2909	GAACUGCU C UUCCUCUU
2608	CUUAGCU C CGGGGGA	2910	ACUUCUU C UCUUUAAC
2609	UGGAGACU A ACUGGAUG	2911	UUCUUCU C UAUUACCC
2620	UCAGAGAU C UGACAGUU	2912	ADGUAUU A UUAUUA
2626	COUCAGU A GCGCGCU	2913	UGOGUAU C GUUCCAG
2628	UACAACAU U UCAGCUOC	2914	GUAIUUUAU U AAUCAGA
2635	UCACAGAU C CAUUCAC	2915	UAUUUAAA A AUUCAGAG
2640	GCUCAGGU A UCCAUCCA	2916	CUUCUCCU C UGGCGAAG
2641	CCCCACCU A CAUACAUU	2917	CUUCCUCU U GCGAAGAC
2642	GCCUGUUU C CGCCUCU	2918	AUUCUUU C ACGAGUCA
2653	CCACAGGU C AGGGUGCU	2919	UUUUGUGU C AGCCACUG
2659	AGAAGGGU C CGCGAGC	2931	GAUGGUGU C CGCGUGCC
2689	ACUAGGGU C CGAAGCU	2933	UGGAGUCU C CCAGCACC
2691	UCAGGOCU A AGAGGACU	2941	CAGUACUU C CCCCAGGC
2700	AGGGUACU U CCCCCAGG	2951	ACCAUCU U CCUCUGAC
2704	GACCACCU C CCCACCUA	2952	CGGACUU U CGAUCUUC
2711	COUCACCU U AGGAAGGU	2955	UGCTUCCU C UGACAUUGG
2712	OCUACCUU A GGAAGGUG	2956	CUUUCUU U GAAUCAAU
2721	GGAAAGAU C AUACGGGU	2961	UUUUGUGU C AGCCACUG
2724	AAGAUCAU A CGGGUJUG	2962	UGUGUAUU C GUUCCAG
2744	GGGUGGGAU C CGUGCAGG	2965	CUUUGAAAU C AAUAAAGU
2750	GUCCUGU U UAAAAACC	2966	UGGAAGCU C UUCAAGCU
2759	GACGAACU A UCGAGUGG	2969	GAUCAAU A AAGUUUUA

2975 UGGAAGCU C UUCAAGCU
2976 UAUAUUGGU C CCACCCUG
2977 GAAGCUCU U CAAGCUGA

Table 10: Rat ICAM HH Ribozyme Sequences

nt. Position	Rat HH Ribozyme Sequence
11	UCAGUGUG CUGAUGAGGCCGAAAGCCGAA AUUUGAUU
23	UAGAGAAAG CUGAUGAGGCCGAAAGCCGAA AAGUCAGC
26	AAGAGGAA CUGAUGAGGCCGAAAGCCGAA AGCHGUUC
31	AGGACCAAG CUGAUGAGGCCGAAAGCCGAA AGCAGAGG
34	GUUAUUCU CUGAUGAGGCCGAAAGCCGAA AGCUUCAG
40	GGGGCJUG CUGAUGAGGCCGAAAGCCGAA ACCUJUGAG
48	CCCAGGCC CUGAUGAGGCCGAAAGCCGAA AGGUUCUC
54	GGCUCAGG CUGAUGAGGCCGAAAGCCGAA AGGCAGGGG
58	GGGAGCTA CUGAUGAGGCCGAAAGCCGAA AGGCACGG
64	AOGGGUUG CUGAUGAGGCCGAAAGCCGAA AGCCAUUG
96	AGGACCAAG CUGAUGAGGCCGAAAGCCGAA AGCAGAGG
102	GOGACCAAG CUGAUGAGGCCGAAAGCCGAA ACCAGGAG
108	AGUUCCCC CUGAUGAGGCCGAAAGCCGAA AGCAGUCC
115	UGGGAAACA CUGAUGAGGCCGAAAGCCGAA AGGUAGGA
119	GAGUUGGG CUGAUGAGGCCGAAAGCCGAA ACAGUGUC
120	GGCCCGGG CUGAUGAGGCCGAAAGCCGAA ADCAACAC
146	GGAGUUCU CUGAUGAGGCCGAAAGCCGAA AGGUUCUG
152	UUGAGGUG CUGAUGAGGCCGAAAGCCGAA AGCCGGGU
158	UGACTUCU CUGAUGAGGCCGAAAGCCGAA AAAAGAAAU
165	GGGGGAAG CUGAUGAGGCCGAAAGCCGAA ACUJGUCA
168	CGAGGCAG CUGAUGAGGCCGAAAGCCGAA AAGGCUUC
185	CCUGGCACG CUGAUGAGGCCGAAAGCCGAA ADCCACCC
209	GGUCAGAU CUGAUGAGGCCGAAAGCCGAA AGGGCUG
227	UUCAACGU CUGAUGAGGCCGAAAGCCGAA ACUUGGUC
230	CCUCCACAC CUGAUGAGGCCGAAAGCCGAA ACAGCUUG
237	GGGGUGUC CUGAUGAGGCCGAAAGCCGAA AGCUUCAG
248	U CCTAAAGG CUGAUGAGGCCGAAAGCCGAA AGGGGCC
253	CCUCCACTU CUGAUGAGGCCGAAAGCCGAA AGGCAGUG
263	GCAGUGAGA CUGAUGAGGCCGAAAGCCGAA AUUUGCU
267	CGAGGCAG CUGAUGAGGCCGAAAGCCGAA AAGGCUUC
293	UCAGCUUG CUGAUGAGGCCGAAAGCCGAA AGAGCUUC
319	UCCUUUUGU CUGAUGAGGCCGAAAGCCGAA AUCCUCG
335	AGUUCUCA CUGAUGAGGCCGAAAGCCGAA AGCACAGU
337	GAGGACCA CUGAUGAGGCCGAAAGCCGAA AUAGCACA
338	CUCAGCUU CUGAUGAGGCCGAAAGCCGAA AAGAGCUU
359	AAGCCGAG CUGAUGAGGCCGAAAGCCGAA ACTUGCGUG
367	ACGGGUUG CUGAUGAGGCCGAAAGCCGAA AGCCAUUG
374	AGGUGGGUJ CUGAUGAGGCCGAAAGCCGAA AGGGGUAA
375	GAGGCAGG CUGAUGAGGCCGAAAGCCGAA AGGCUTUC
378	UACCCUGU CUGAUGAGGCCGAAAGCCGAA AGGUGGGU
386	AGCUCCAA CUGAUGAGGCCGAAAGCCGAA ACACAGCG

394 CUGUUCAG CUGAUGAGGCCGAAAGGCGAA AGCACCCAC
 420 UGGCGUGG CUGAUGAGGCCGAAAGGCGAA AGGGGUGC
 425 GGUGGCAG CUGAUGAGGCCGAAAGGCGAA AGCCGAGG
 427 UGGUUUUU CUGAUGAGGCCGAAAGGCGAA AACAGGGA
 450 CGCAGGAA CUGAUGAGGCCGAAAGGCGAA AGGUUCUU
 451 GCCUGGGG CUGAUGAGGCCGAAAGGCGAA AAGUACCC
 456 UGGUGGCA CUGAUGAGGCCGAAAGGCGAA AAGCCGAG
 495 UACACAGU CUGAUGAGGCCGAAAGGCGAA AUUGGUGC
 510 UCCCCACG CUGAUGAGGCCGAAAGGCGAA AGCAGCAC
 564 GUGGUUGG CUGAUGAGGCCGAAAGGCGAA ACACUUUC
 592 UCCCCUGGJ CUGAUGAGGCCGAAAGGCGAA AUACUCCC
 607 GCAGDAGA CUGAUGAGGCCGAAAGGCGAA AUUGGUC
 608 AGCAUGAG CUGAUGAGGCCGAAAGGCGAA AUUUGGU
 609 AAGCAUGA CUGAUGAGGCCGAAAGGCGAA AAAUUGGC
 611 UGAAGCAU CUGAUGAGGCCGAAAGGCGAA AGAAAATUG
 CAIUCUUG CUGAUGAGGCCGAAAGGCGAA ACAGUGAC
 657 ACAUUCU CUGAUGAGGCCGAAAGGCGAA AACAGUGA
 668 AAGAGGAA CUGAUGAGGCCGAAAGGCGAA AGCAGUUC
 677 UGGCGUGG CUGAUGAGGCCGAAAGGCGAA AGGGGUGC
 684 AAAGUCCG CUGAUGAGGCCGAAAGGCGAA AGCUUGGU
 692 CGAGGUUC CUGAUGAGGCCGAAAGGCGAA AGGUUGGG
 693 GGAAGAAC CUGAUGAGGCCGAAAGGCGAA AAAGUCCG
 696 AGAGGCAG CUGAUGAGGCCGAAAGGCGAA AAACAGGC
 709 GUGAGGGG CUGAUGAGGCCGAAAGGCGAA AAAUGCUG
 720 GAGCTUGAA CUGAUGAGGCCGAAAGGCGAA AGUUGUAG
 723 UGGGAGCU CUGAUGAGGCCGAAAGGCGAA AAAAGUUG
 735 GOGACCAG CUGAUGAGGCCGAAAGGCGAA ACCAGGAG
 738 UCCACCCC CUGAUGAGGCCGAAAGGCGAA AGGCAGGA
 765 AGUUCUCA CUGAUGAGGCCGAAAGGCGAA AGCACAGU
 769 UUCCAGGG CUGAUGAGGCCGAAAGGCGAA ACACAAGA
 770 CUUCCAGG CUGAUGAGGCCGAAAGGCGAA AACACRAAG
 785 AGGCAGGA CUGAUGAGGCCGAAAGGCGAA ACAGGCCU
 786 GAGGCAGG CUGAUGAGGCCGAAAGGCGAA AACAGGCC
 792 GOGACCAG CUGAUGAGGCCGAAAGGCGAA ACCAGGAG
 794 GAGCTUCA CUGAUGAGGCCGAAAGGCGAA AGGCAGGA
 807 UCCAGGUA CUGAUGAGGCCGAAAGGCGAA AUUCUGAGC
 833 UAGUCUCC CUGAUGAGGCCGAAAGGCGAA ACCCCAGG
 846 CAATAAAAU CUGAUGAGGCCGAAAGGCGAA ACUGUCAG
 851 AGCUUGCUA CUGAUGAGGCCGAAAGGCGAA AGGUGAGC
 863 ACGGGUUG CUGAUGAGGCCGAAAGGCGAA AACCAUUG
 866 UGUCAGAG CUGAUGAGGCCGAAAGGCGAA AAGCAUGG
 867 UAGGUGGG CUGAUGAGGCCGAAAGGCGAA AGGUUGUC
 869 CUUOGCAA CUGAUGAGGCCGAAAGGCGAA AGGAAGAG
 881 CACGGGUU CUGAUGAGGCCGAAAGGCGAA AAGCCAUU
 885 UUCACAGU CUGAUGAGGCCGAAAGGCGAA ACUUGGU
 933 CGGGGAAC CUGAUGAGGCCGAAAGGCGAA AAUACACA
 936 UGACACAA CUGAUGAGGCCGAAAGGCGAA AUUCUGC
 978 AAGUUGUA CUGAUGAGGCCGAAAGGCGAA AUUCUCAA
 980 AAAAGUUG CUGAUGAGGCCGAAAGGCGAA AGAUUCUC

986 GAGCTUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUAG
 987 GGAGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUUGUA
 988 GGGAGCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUUGU
 1005 GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAACGAA
 1006 CCUGGGUGA CUGAUGAGGCCGAAAGGCCGAA ACUCCCAC
 1023 CCTTUCUGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCCG
 1025 CCCCTUCU CUGAUGAGGCCGAAAGGCCGAA AGACCTTC
 1066 UGGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
 1092 UCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCTCTU
 1093 AGGGGCUG CUGAUGAGGCCGAAAGGCCGAA ACUCCCCU
 1125 AUCAACAA CUGAUGAGGCCGAAAGGCCGAA AGUUGGGG
 1163 AGCRAAAG CUGAUGAGGCCGAAAGGCCGAA AGCGUOGU
 1164 GAGCAAA CUGAUGAGGCCGAAAGGCCGAA AAGGUGCG
 1166 CAGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGCGU
 1172 AGGCCGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAAG
 1200 UUCAGOGU CUGAUGAGGCCGAAAGGCCGAA AUUUGGAAU
 1201 CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCAA
 1203 GACCTUGG CUGAUGAGGCCGAAAGGCCGAA AGAAGCCC
 1227 AGCACAUG CUGAUGAGGCCGAAAGGCCGAA AGUUCCAA
 1228 ACGAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGCCCGC
 1233 GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAGGAG
 1238 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGGACA
 1264 ACCCGTAAU CUGAUGAGGCCGAAAGGCCGAA ACCUUUUC
 1267 CAUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGUGAC
 1294 CUGACACA CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
 1295 UCCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUCU
 1306 GCAUGUTAA CUGAUGAGGCCGAAAGGCCGAA AGUCUGCU
 1321 UUUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUGUU
 1334 GCUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGAUATAC
 1344 GGAAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCRAACGA
 1351 AGUUCUCU CUGAUGAGGCCGAAAGGCCGAA AGGCUCUGA
 1353 CCAUJUGU CUGAUGAGGCCGAAAGGCCGAA AGCTUGCUA
 1366 CCTUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
 1367 GCGUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 1368 GGCAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACCAJC
 1380 ACCAUCCCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCAG
 1388 CAUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUCUCCA
 1398 UGUCCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGCCAG
 1402 CAGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACAG
 1408 GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAACGAA
 1410 GUCCACTUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUCG
 1421 GCGUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 1425 AGCCAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUUGGU
 1429 CCTUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAGUAU
 1444 CUCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUCU
 1455 UCCUGGGU CUGAUGAGGCCGAAAGGCCGAA AUACUCCC
 1482 CCTUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
 1484 GCAAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAGU
 1493 UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG

1500 UUGACCAU CUGAUGAGGCGAAAGGCCGAA AUUUCACG
 1503 GUGGUJGG CUGAUGAGGCGAAAGGCCGAA ACAUUUUC
 1506 CCAACAAU CUGAUGAGGCGAAAGGCCGAA ADGACCCA
 1509 UACACAGU CUGAUGAGGCGAAAGGCCGAA ADGGJGGC
 1518 ACRACGGC CUGAUGAGGCGAAAGGCCGAA ACCAGGAC
 1530 ACAAUUAU CUGAUGAGGCGAAAGGCCGAA ACCCAGGU
 1533 AAGCCCGC CUGAUGAGGCGAAAGGCCGAA AUGAUCAG
 1551 UACGAGCA CUGAUGAGGCGAAAGGCCGAA AGGGCCAC
 1559 UAAACAGG CUGAUGAGGCGAAAGGCCGAA ACUUCCCA
 1563 UGGGAACA CUGAUGAGGCGAAAGGCCGAA AGGUAGGA
 1565 GGGGUAAA CUGAUGAGGCGAAAGGCCGAA AGGUUAAA
 1567 CUUGCCGU CUGAUGAGGCGAAAGGCCGAA AUAGGUGU
 1584 UAUADCCU CUGAUGAGGCGAAAGGCCGAA ADCUUCCU
 1592 GUAACTUUG CUGAUGAGGCGAAAGGCCGAA AUADUCCUG
 1599 GCCUUCUG CUGAUGAGGCGAAAGGCCGAA AACUUGUA
 1651 GGCUCAGG CUGAUGAGGCGAAAGGCCGAA AGGGGGGG
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 1680 GACCUUGG CUGAUGAGGCGAAAGGCCGAA AGAAGCCC
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 1684 GAGAGGUC CUGAUGAGGCGAAAGGCCGAA ACGAGCAG
 1690 AAUGUAUG CUGAUGAGGCGAAAGGCCGAA AGGUUGGG
 1691 GAAGAUOG CUGAUGAGGCGAAAGGCCGAA AAGUCCGG
 1696 GCGACCAG CUGAUGAGGCGAAAGGCCGAA ACCAGGAG
 1698 UCUCUAGG CUGAUGAGGCGAAAGGCCGAA AUADUGA
 1737 GCACCGUG CUGAUGAGGCGAAAGGCCGAA ADGUGAUC
 1750 AAUAGGUG CUGAUGAGGCGAAAGGCCGAA AAAUGGAC
 1756 AGGACCAAG CUGAUGAGGCGAAAGGCCGAA AGCAGAGG
 1787 COCAGGCC CUGAUGAGGCGAAAGGCCGAA AGGUUCUC
 1790 GAGUUGGG CUGAUGAGGCGAAAGGCCGAA ACAGUGUC
 1793 GUCCAGGU CUGAUGAGGCGAAAGGCCGAA AGGACCAU
 1797 UGGUUUUU CUGAUGAGGCGAAAGGCCGAA AACAGGGA
 1802 UCCAGGU A CUGAUGAGGCGAAAGGCCGAA ADUUGAGC
 1812 UUUCCCCA CUGAUGAGGCGAAAGGCCGAA ACUCUGUU
 1813 ACGAUCAC CUGAUGAGGCGAAAGGCCGAA AAGCCCGC
 1825 UACACAGU CUGAUGAGGCGAAAGGCCGAA ADGGUGGC
 1837 UACCCUGU CUGAUGAGGCGAAAGGCCGAA AGGUUGGG
 1845 GGGCCUCC CUGAUGAGGCGAAAGGCCGAA AGUOCUCU
 1856 GCAGGUCA CUGAUGAGGCGAAAGGCCGAA ADUAGGGG
 1861 GGACCAUA CUGAUGAGGCGAAAGGCCGAA AGCACAUG
 1865 CUUGUGUC CUGAUGAGGCGAAAGGCCGAA ACCGGAUU
 1868 AUUUAUAU CUGAUGAGGCGAAAGGCCGAA ACUCUGUA
 1877 CCUGGGGG CUGAUGAGGCGAAAGGCCGAA AGUACUGU
 1901 UGUACCUU CUGAUGAGGCGAAAGGCCGAA AGUUUUAG
 1912 UGUCCAUU CUGAUGAGGCGAAAGGCCGAA AUUCUGUUC
 1922 UAGGCRAU CUGAUGAGGCGAAAGGCCGAA ACUUAUAC
 1923 CTAAAGGU CUGAUGAGGCGAAAGGCCGAA AGCGUCCA
 1928 UCCAGGU A CUGAUGAGGCGAAAGGCCGAA AUUCUGAGC

1930 CAUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUCUCCA
 1964 GCTGACAC CUGAUGAGGCCGAAAGGCCGAA AAAUCUCU
 1983 CCCAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCUC
 1996 AGCUUUGAA CGGAGGAGGCCGAAAGGCCGAA AGCUUCCA
 2005 UAGGCAAU CUGAUGAGGCCGAAAGGCCGAA ACUUTACAU
 2013 CAUCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGCG
 2015 ACCAUCCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCAG
 2020 GUACAGGG CUGAUGAGGCCGAAAGGCCGAA ACUCAATA
 2039 UGGUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCGG
 2040 ACCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUUCAG
 2057 AGCCAUUG CUGAUGAGGCCGAAAGGCCGAA AGGACCAAG
 2061 UAGGUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGAOGC
 2071 CCUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGTAT
 2076 UUAGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGCUCACA
 2097 ACAUCAAC CUGAUGAGGCCGAAAGGCCGAA AGAGUUGG
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 2115 CAGGACCC CUGAUGAGGCCGAAAGGCCGAA AGUOOGAA
 2128 GAUCAUUG CUGAUGAGGCCGAAAGGCCGAA ACAGCACU
 2130 AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
 2145 ACADCAAC CUGAUGAGGCCGAAAGGCCGAA AGAGUUGG
 2152 AAGUUGUA CUGAUGAGGCCGAAAGGCCGAA AUUCUCAA
 2156 UCAUAAA CUGAUGAGGCCGAAAGGCCGAA AACUGUCA
 2158 AAUUAATA CUGAUGAGGCCGAAAGGCCGAA AUACACCA
 2159 GAUUAATA CUGAUGAGGCCGAAAGGCCGAA AAUACAUU
 2160 UGAUUTAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
 2162 AACAAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAADGU
 2163 CUUGAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAATA
 2166 AAUUAATA CUGAUGAGGCCGAAAGGCCGAA AUACACCA
 2167 GAUUAATA CUGAUGAGGCCGAAAGGCCGAA AAUACACU
 2170 UCUGAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAACAC
 2171 UACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAACUG
 2173 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
 2174 AGCACGGG CUGAUGAGGCCGAAAGGCCGAA AAUAGAGA
 2175 UGACUCUGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAA
 2176 GUGGUUGG CUGAUGAGGCCGAAAGGCCGAA ACUUTUUC
 2183 UCAUAAA CUGAUGAGGCCGAAAGGCCGAA AACUGUCA
 2185 ACUCAATA CUGAUGAGGCCGAAAGGCCGAA AUUACUGU
 2186 UACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAACTG
 2187 GUACUCAA CUGAUGAGGCCGAAAGGCCGAA AAUUAACU
 2189 GGGUACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAATA
 2196 CAUAAAATA CUGAUGAGGCCGAAAGGCCGAA ACUGUCAG
 2198 UGACCUUG CUGAUGAGGCCGAAAGGCCGAA AGACACUC
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 2224 AAGGUAGG CUGAUGAGGCCGAAAGGCCGAA AUGUAUGU

2226 UGUGGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCAG
 2233 AGUUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUUA
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 2248 GOGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 2254 UUCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
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 2260 AGCACCGU CUGAUGAGGCCGAAAGGCCGAA AUUGUGAU
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 2279 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA ADCUUUCC
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 2288 CAUUGGAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGGC
 2291 GUAACTUUG CUGAUGAGGCCGAAAGGCCGAA AUUUCUUG
 2321 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA ADCUUUCC
 2338 CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCAA
 2339 GGCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 2341 UGAGCACC CUGAUGAGGCCGAAAGGCCGAA ACAGCCCC
 2344 GAGAGGUC CUGAUGAGGCCGAAAGGCCGAA ACGAGCAG
 2358 UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
 2359 UUCUGUGG CUGAUGAGGCCGAAAGGCCGAA ADGGAUUG
 2360 CUUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACACAAG
 2376 AAGAGGA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
 2377 UAAUAGAG CUGAUGAGGCCGAAAGGCCGAA AGGAAGUC
 2378 UCGUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAGC
 2379 CGCAAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGCAG
 2380 ACUUCUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAUC
 2382 UGACUCGU CUGAUGAGGCCGAAAGGCCGAA AAGAAA
 2384 CUUGUGUC CUGAUGAGGCCGAAAGGCCGAA ACCGGAUU
 2399 CGUCCACA CUGAUGAGGCCGAAAGGCCGAA AGUAAUUA
 2401 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAAGCACA
 2411 UGAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAA
 2417 AACUUGUA CUGAUGAGGCCGAAAGGCCGAA ACCCTUGAU
 2418 AGUUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUUA
 2425 GAACUCUG CUGAUGAGGCCGAAAGGCCGAA AUUAA
 2426 UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG
 2433 AACUGUCA CUGAUGAGGCCGAAAGGCCGAA AACUCUGA
 2434 UCGUUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCCG
 2448 GGGGGAAG CUGAUGAGGCCGAAAGGCCGAA ACCGUUCA
 2449 CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
 2451 GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGCC
 2452 AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
 2455 AACAAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAAGGU
 2459 UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
 2460 UGGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
 2479 GCGGGUAA CUGAUGAGGCCGAAAGGCCGAA AGGUUGUA
 2480 GGGAUACAC CUGAUGAGGCCGAAAGGCCGAA ACGGCAC
 2483 ACAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACGAAGGU
 2484 GACAUUUGG CUGAUGAGGCCGAAAGGCCGAA AACAAAGG
 2492 UAGGUUGG CUGAUGAGGCCGAAAGGCCGAA AGGUUGUC

2504 UAGGAADG CUGAUGAGGCCGAAAGGCGAA AUGUAGGU
 2508 AAGGUAGG CUGAUGAGGCCGAAAGGCGAA AUGUAGGU
 2509 AAAGGJAG CUGAUGAGGCCGAAAGGCGAA ADGUADG
 2510 AADAGGUG CUGAUGAGGCCGAAAGGCGAA AAAUGGAC
 2520 ACATUCCG CUGAUGAGGCCGAAAGGCGAA ACAAAGGU
 2521 GACAUUCC CUGAUGAGGCCGAAAGGCGAA AACAAAGG
 2533 UGAGGGGU CUGAUGAGGCCGAAAGGCGAA ADGCUGU
 2540 GCAUACCU CUGAUGAGGCCGAAAGGCGAA AGCAACGA
 2545 AAAGUCCG CUGAUGAGGCCGAAAGGCGAA AGCUGCCU
 2568 CUGACACA CUGAUGAGGCCGAAAGGCGAA AADUCUG
 2579 CCAGGGCA CUGAUGAGGCCGAAAGGCGAA AGUGCAGG
 2585 GAGAGGUC CUGAUGAGGCCGAAAGGCGAA ACCGAGCAG
 2588 GGCUGUGG CUGAUGAGGCCGAAAGGCGAA AGGAGGCA
 2591 CTUUCGCAA CUGAUGAGGCCGAAAGGCGAA AGGAAGAG
 2593 AGCAGGGG CUGAUGAGGCCGAAAGGCGAA AATAGAGA
 2596 GCGACCCAG CUGAUGAGGCCGAAAGGCGAA ACCRAGGAG
 2601 GAGGACCA CUGAUGAGGCCGAAAGGCGAA ATAGCACA
 2602 ACAACGGC CUGAUGAGGCCGAAAGGCGAA ACCAGGAC
 2607 CCUGGUGA CUGAUGAGGCCGAAAGGCGAA ACUCCCCAC
 2608 UOOCACGG CUGAUGAGGCCGAAAGGCGAA AGCTAAAG
 2609 CAUCCAGU CUGAUGAGGCCGAAAGGCGAA AGUCUCCA
 2620 AACUGUCA CUGAUGAGGCCGAAAGGCGAA AACUCUGA
 2626 AGCAGCAC CUGAUGAGGCCGAAAGGCGAA ACUGAGAG
 2628 GGAGCUGA CUGAUGAGGCCGAAAGGCGAA AAGUUGUA
 2635 GUGAAUUG CUGAUGAGGCCGAAAGGCGAA ADCUGUGA
 2640 UGGADGGA CUGAUGAGGCCGAAAGGCGAA ACCUGAGC
 2641 AADGUADG CUGAUGAGGCCGAAAGGCGAA AGGUUGGG
 2642 AGAGGCAAG CUGAUGAGGCCGAAAGGCGAA AAACAGGC
 2653 AGCACCCU CUGAUGAGGCCGAAAGGCGAA ACCUGUGG
 2659 GCUCUGCAG CUGAUGAGGCCGAAAGGCGAA ACCUUUCU
 2689 AGCTTUCAG CUGAUGAGGCCGAAAGGCGAA ACCUJAGU
 2691 AGUCCUCU CUGAUGAGGCCGAAAGGCGAA AGGCCUGA
 2700 CCUGGGGG CUGAUGAGGCCGAAAGGCGAA AGUACCCU
 2704 TAGGUGGG CUGAUGAGGCCGAAAGGCGAA AGGUUGGC
 2711 ACCUUCU CUGAUGAGGCCGAAAGGCGAA AGGUAGGG
 2712 CACCTUCC CUGAUGAGGCCGAAAGGCGAA AAGGUAGG
 2721 ACCGUAU CUGAUGAGGCCGAAAGGCGAA ADCUUUCCC
 2724 CAAACCG CUGAUGAGGCCGAAAGGCGAA AUGADCUU
 2744 CCUGCAAG CUGAUGAGGCCGAAAGGCGAA ADCACCCC
 2750 GGTTTTTA CUGAUGAGGCCGAAAGGCGAA ACAGGGAC
 2759 CCACUCGA CUGAUGAGGCCGAAAGGCGAA AGUUCGUC
 2761 GGAAGAUC CUGAUGAGGCCGAAAGGCGAA AAAGUCCG
 2765 AGGCGCA CUGAUGAGGCCGAAAGGCGAA AGCAAAAG
 2769 GCAGGGGU CUGAUGAGGCCGAAAGGCGAA AATAGAGAA
 2797 UUGACCAU CUGAUGAGGCCGAAAGGCGAA AUUUCACG
 2803 GUUCUGUG CUGAUGAGGCCGAAAGGCGAA AGCAGGAG
 2804 AGUUCUGU CUGAUGAGGCCGAAAGGCGAA AAGCAUGA
 2813 AGGGUCAG CUGAUGAGGCCGAAAGGCGAA AUGGGAGC
 2815 GGAAGAUC CUGAUGAGGCCGAAAGGCGAA AAAGUCCG

2821 ACCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCAAG
 2822 GGAGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUUGUA
 2823 UGGGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAAGUUG
 2829 GGAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCACCGA
 2837 GGGGAAAG CUGAUGAGGCCGAAAGGCCGAA ACCCUCGG
 2840 UGGGCTUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUGC
 2847 AGGUGGGU CUGAUGAGGCCGAAAGGCCGAA AGGGGUAA
 2853 CUAGUCGG CUGAUGAGGCCGAAAGGCCGAA AGAUCCGA
 2860 UUCCAGGG CUGAUGAGGCCGAAAGGCCGAA ACACAAAGA
 2872 UGAGCACC CUGAUGAGGCCGAAAGGCCGAA ACAGGGCCC
 2877 GGUGCTUGG CUGAUGAGGCCGAAAGGCCGAA AGACUCCA
 2899 AAAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGCTUGCC
 2900 AGAGAAAG CUGAUGAGGCCGAAAGGCCGAA AGUCAGCC
 2904 AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAAGUCC
 2905 AGAGAAAG CUGAUGAGGCCGAAAGGCCGAA AGUCAGCC
 2906 UUAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACACUCAAC
 2907 CGCAAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGCAG
 2908 AAUUAUAA CUGAUGAGGCCGAAAGGCCGAA AUACAUCA
 2909 AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
 2910 GUAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAAGJ
 2911 GGGTAUAA CUGAUGAGGCCGAAAGGCCGAA AGAAGGAA
 2912 UGAUAAUAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
 2913 CGGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAUACACA
 2914 UCUGAAAU CUGAUGAGGCCGAAAGGCCGAA AUAAAUAC
 2915 CUUUGCAA CUGAUGAGGCCGAAAGGCCGAA AAUAAUAA
 2916 GUUUUOGC CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
 2917 UGACUOGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAAU
 2918 CAGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACACAAAA
 2919 GGCAGCGG CUGAUGAGGCCGAAAGGCCGAA ACACCAUC
 2931 GGUGCTUGG CUGAUGAGGCCGAAAGGCCGAA AGACUCCA
 2941 GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACUG
 2951 GUAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGCAUGGU
 2952 GAAGADOG CUGAUGAGGCCGAAAGGCCGAA AAGUCCGG
 2955 CCAUGUCA CUGAUGAGGCCGAAAGGCCGAA AGGAAGCA
 2956 AUUGAUUC CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
 2961 CAGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACACAAAA
 2962 CGGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAUACACA
 2965 ACUUAUAA CUGAUGAGGCCGAAAGGCCGAA AUUCAAAG
 2966 AGCUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCUUCCA
 2969 UAAAACUU CUGAUGAGGCCGAAAGGCCGAA AUUGAUUC
 2975 AGCUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCUUCCA
 2976 CAGGUGAG CUGAUGAGGCCGAAAGGCCGAA ACCAUATA
 2977 UCAGCTUG CUGAUGAGGCCGAAAGGCCGAA AGAGCUUC

Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	ADGCACU U UCUUUGC	245	AAGAAAU C UUUCAGG
9	UGCACUU U CCUUGCC	247	GAAACTJ U UCAGGGAA
10	GCACUUU C UUUGCCA	248	AAAUCUU U CAGGGAA
12	ACUUCU U UGCCAAA	249	AAUCUUU C AGGGAAU
13	CUUUCU U GCCAAAG	257	AGCGAAU A GGCACAC
36	AGAACGU U UCAAGGC	273	GGAGAGU C AAACUGU
37	GAACGU U CAGAGCC	291	AGCCCCGU A CUGUGGA
38	AACGCUU C AGAGCCA	305	AAAGACU A UUCAAA
56	GGADGCU U CUGCAUU	307	AGACUAU U CAAAAAC
57	GAUGCUU C UGCAUUU	308	GACUADU C AAAAACU
63	UCUGCAU U UGAGUUU	316	AAAAACU U GUCCUUA
64	CUGCAUU U GAGUUUG	319	AACUUGU C CUAAAUA
69	UUGAGGU U UGCUAGC	322	UUGUCCU U AAUAAAAG
70	UUGAGGU U GCUGACU	323	UGUCCUU A AUAAAAGA
74	GUUUGCU A GCUCUUG	326	CCUUAUU A AAGAAAU
78	GCTAGCU C UUGGAGC	334	AAGAAAU A CAUUGAC
80	UAGCTCU U GGAGCUG	338	AAUTACAU U GACGGCC
91	GCUGGCCU A CGUGUAU	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCADC	388	AACCAAU U CCUAGAC
104	AUGCCAU C CCCACAG	389	ACCAAUU C CUAGACU
116	CGAAAUA U CCCACAA	392	AAUUCCU A GACUACC
117	AGAAAUA C CCACRAAG	397	CTAGACU A CCUGCAA
130	AGUGCAU U GGCGAAA	409	CAAGAGU U UCUUGGU
145	GAGACCU U GGACACUG	410	AAGAGUU U CUUGGUG
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGCUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUUGCUU C UACUCAU	419	UUGGUGU A AUGAAC
159	GCUUCU A CUCACUG	437	AGUGGAAU A AUAGAAA
162	UUCUACU C ADGGAAC	440	GGAAUAAU A GAAAGGU
165	UACUCAU C GAACCU	447	AGAAAGU U GAGACTA
171	UCGAACU C UGCUGAU	454	UGAGACU A AACUGGU
179	UGCUGAU A GCCAAGG	462	AACTGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGAU U CCUGGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CGUUCC	479	CAAAGAU U UGGAGGG
206	UUCUGGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CGUACAC	481	AAGAUUU U GGAGGGAG
212	UUCUGGU A CAUAAA	497	AGGACAU U UUACUGC
216	UGUACAU A AAAAUCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACU	499	GACAUUU U ACUGCAG

500	ACAUUUU A CGCGAGU	684	UACUUUU U UCUCUAUU
531	AAAGAGU C AGGCCUU	685	ACUUUUU U CUUUAUUU
538	CAGGCCU U AAUUUUC	686	CUUUUUU C UUAAUUA
539	AGGCCUU A ADUUUCA	688	UUUUUCU U AUUUAAC
542	CCUUUAAU U UUCAADA	689	UUUUCCU A UUUAACU
543	CUUAAAU U UCAAAU	691	UUCUUAU U UAACUUA
544	UCAAAUU U CAUAAU	692	UCUUAU U AACUUA
545	UAAUUUU C AAUAA	693	CUUAAU A ACUUAAC
549	UUUCAAU A UUADU	697	UUUAACU U AACAUUC
551	UCAAAU A ADUUAAC	698	UUAACU A ACAUUCU
554	AUAAAUU U UAACUUC	703	UUAACAU U CGUAAA
555	UAAUAAU U AACUUA	704	UAAACAU C UGAAA
556	AUAAUUU A ACUUCAG	708	AUUCUGU A AAAUGUC
560	UUUAACU U CRAGAGGG	715	AAAADGU C UGUAAC
561	UUAACUU C AGAGGGA	719	UGUCUGU U AACUUA
573	GGAAAGU A AAIAUUU	720	GUUCUGU A ACUUAU
577	AGUAAA U UUUCAGG	724	GUUAACU U AAUAGUA
579	UAAAUAU U UCAGGCA	725	UUAACU A AAUAGAU
580	AAUAAAU U CAGGCAC	728	ACUUAU A GUAAUUA
581	AAIAUUU C AGGCATA	731	UAADGU A UUUAUGA
588	CRGGCAU A CUGACAC	733	UAGUAU U UADGAAA
597	UGACACU U UGOCAGA	734	UAGUAU U AUGAAAU
598	GACACOU U GCGAGAA	735	AGUADUU A UGAAADG
611	AAAGCAU A AAADCU	745	AAADGGU U AAGAADU
616	AUAAAAU U CUUAAA	746	AAUGGUU A AGAAUJU
617	UAAAADU C UUAAAU	752	UAGAAAU U UGGAAA
619	AAAUCU U AAAAU	753	AAGAAU U GGAAA
620	AAUUCUU A AAADADA	757	AAUUGGU A AAUAGU
625	UAAAADU A UAUUCA	761	GGUAAA U AGUAAU
627	AAAADAU A UUUCAGA	762	GUAAADU A GUAAUUA
629	AAIAAUU U UCAGADA	765	AAUAGU A UUUAUU
630	AUAAAUU U CAGAAU	767	UUAGUAU U UAUUAA
631	UAAUAAU C AGAAUAC	768	UAGUADU U AUUUAU
636	UUCAGAU A UCAGAAU	769	AGUADUU A UUUAUG
638	CGAAUAU C AGAAUCA	771	UAAUUAU U UAAADGU
644	UCAGAAU C AUUGAAG	772	AUUUAU U AAUGUUA
647	GAACAU U GAAGAU	773	UUUAAU A ADGUUAU
653	UOGAAGU A UUUCUCU	778	UUAADGU U ADGUOGU
655	GAAGUAU U UUCCUCC	779	UAAADGU A UGUOGUG
656	AAGUADU U UCUCCCA	783	GUADGU U GUGUUCU
657	AGUADUU U CCUCAG	788	GUUGUGU U CUAAUAA
658	GUAUUUU C CCUCAGG	789	UUGUGUU C UUAAUAA
661	UUUUCU C CAGGCAA	791	GUUGUCU A AUAAAAC
672	GCIAAAAU U GAAUAC	794	UUCUAAU A AAACAAA
676	AAUUGAU A UACTUUU	805	CAAAAU A GACAACU
678	UUGAUAU A CUUUUUU		
681	AUAAACU U UUUUCUU		
682	UAAUACU U UUUCUUA		

Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GC _{AAAGA} CUGAU _{GAGGCCGAAAGGCCGAA} AGGGCAU
9	GGC _{AAAG} CUGAU _{GAGGCCGAAAGGCCGAA} AAGUGCA
10	U _{GGCGAA} CUGAU _{GAGGCCGAAAGGCCGAA} AAAGUGC
12	U _{UUGGCA} CUGAU _{GAGGCCGAAAGGCCGAA} AGAAAGU
13	C _{UUUGGC} CUGAU _{GAGGCCGAAAGGCCGAA} AAGAAAAG
36	GC _{UCUGA} CUGAU _{GAGGCCGAAAGGCCGAA} ACGUUCU
37	GGC _{UCUG} CUGAU _{GAGGCCGAAAGGCCGAA} AACGUUC
38	U _{GGCUCU} CUGAU _{GAGGCCGAAAGGCCGAA} AAACGUU
56	A _{AUGCAG} CUGAU _{GAGGCCGAAAGGCCGAA} AGCAUCC
57	AA _{AUGCA} CUGAU _{GAGGCCGAAAGGCCGAA} AAGCAUC
63	AA _{ACUCA} CUGAU _{GAGGCCGAAAGGCCGAA} AUGCAGA
64	CA _{AACUC} CUGAU _{GAGGCCGAAAGGCCGAA} AAUGCAG
69	GC _{UAGCA} CUGAU _{GAGGCCGAAAGGCCGAA} ACUCAAA
70	AG _{CTUAGC} CUGAU _{GAGGCCGAAAGGCCGAA} AACUCAA
74	CA _{AGAGC} CUGAU _{GAGGCCGAAAGGCCGAA} AGCAAAC
78	GC _{UCCAA} CUGAU _{GAGGCCGAAAGGCCGAA} AGCUAGC
80	C _{AGCUCC} CUGAU _{GAGGCCGAAAGGCCGAA} AGAGCTA
91	A _{UACACG} CUGAU _{GAGGCCGAAAGGCCGAA} AGGCAGC
97	G _{AGGGCA} CUGAU _{GAGGCCGAAAGGCCGAA} ACAGGTA
104	C _{OUGGGG} CUGAU _{GAGGCCGAAAGGCCGAA} ADGGCAU
116	U _{UGUGGG} CUGAU _{GAGGCCGAAAGGCCGAA} AUUUCUG
117	C _{OUGUGG} CUGAU _{GAGGCCGAAAGGCCGAA} AAUUUCU
130	U _{UUCACC} CUGAU _{GAGGCCGAAAGGCCGAA} AUGCACU
145	C _{AGUGCC} CUGAU _{GAGGCCGAAAGGCCGAA} AGGUUC
155	G _{AGUAGA} CUGAU _{GAGGCCGAAAGGCCGAA} AGCAGUG
156	U _{GAGUAG} CUGAU _{GAGGCCGAAAGGCCGAA} AAGCAGU
157	A _{UGAGUA} CUGAU _{GAGGCCGAAAGGCCGAA} AAAGCAG
159	C _{GAUGAG} CUGAU _{GAGGCCGAAAGGCCGAA} AGAAAGC
162	G _{UUCGAA} CUGAU _{GAGGCCGAAAGGCCGAA} AGUAGAA
165	A _{GAGUUC} CUGAU _{GAGGCCGAAAGGCCGAA} ADGAGUA
171	A _{UCAGCA} CUGAU _{GAGGCCGAAAGGCCGAA} AGUUCGA
179	C _{AUUGGC} CUGAU _{GAGGCCGAAAGGCCGAA} AUCAAGCA
192	A _{UCCUCA} CUGAU _{GAGGCCGAAAGGCCGAA} AGUCUCA
200	G _{AACAGG} CUGAU _{GAGGCCGAAAGGCCGAA} AUCCUCA
201	G _{GAACAG} CUGAU _{GAGGCCGAAAGGCCGAA} AADCCUC
206	G _{UACAGG} CUGAU _{GAGGCCGAAAGGCCGAA} ACAGGAA
207	U _{GUACAG} CUGAU _{GAGGCCGAAAGGCCGAA} AACAGGA
212	U _{UUUUAUG} CUGAU _{GAGGCCGAAAGGCCGAA} ACAGGAA
216	U _{GAUUUU} CUGAU _{GAGGCCGAAAGGCCGAA} AUGUACA
222	A _{GUUGGU} CUGAU _{GAGGCCGAAAGGCCGAA} AUUUUUA
245	C _{CUGAAA} CUGAU _{GAGGCCGAAAGGCCGAA} AUUUCUU

247 UCCCUGA CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
 248 UUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGAUUU
 249 AUUCCCU CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
 257 GUGUGCC CUGAUGAGGCCGAAAGGCCGAA AUUCCCU
 273 ACAGUUU CUGAUGAGGCCGAAAGGCCGAA ACUCUCC
 291 UOCCACAG CUGAUGAGGCCGAAAGGCCGAA ACCCCCCU
 305 UUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGUCUUU
 307 GUUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGUCU
 308 AGUUUUU CUGAUGAGGCCGAAAGGCCGAA AAUTAGUC
 316 UAAGGAC CUGAUGAGGCCGAAAGGCCGAA AGUUUUU
 319 UAUUAAG CUGAUGAGGCCGAAAGGCCGAA ACAAGUU
 322 CUUUAUU CUGAUGAGGCCGAAAGGCCGAA AGGACAA
 323 UCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAGGACA
 326 AUUUCUU CUGAUGAGGCCGAAAGGCCGAA AUUAAGG
 334 GUCAADG CUGAUGAGGCCGAAAGGCCGAA AUUUCUU
 338 GGGCGUC CUGAUGAGGCCGAAAGGCCGAA AUGUAUU
 380 AUUGGUU CUGAUGAGGCCGAAAGGCCGAA ACUCUCC
 388 GUOAGG CUGAUGAGGCCGAAAGGCCGAA AUUGGUU
 389 AGUCUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
 392 GGUAGUC CUGAUGAGGCCGAAAGGCCGAA AGGAJUU
 397 UUGCAGG CUGAUGAGGCCGAAAGGCCGAA AGUCUAG
 409 ACCRAGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
 410 CACCAAG CUGAUGAGGCCGAAAGGCCGAA AACUCUU
 411 ACACCAA CUGAUGAGGCCGAAAGGCCGAA AAACUCU
 413 UTACACC CUGAUGAGGCCGAAAGGCCGAA AGAAAATU
 419 UGUUCAU CUGAUGAGGCCGAAAGGCCGAA ACACCAA
 437 UUUCUAU CUGAUGAGGCCGAAAGGCCGAA AUCCACU
 440 AACUUUC CUGAUGAGGCCGAAAGGCCGAA AUUAUCC
 447 TAGUCUC CUGAUGAGGCCGAAAGGCCGAA ACUUUCU
 454 ACCAGUU CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
 462 UGCAACA CUGAUGAGGCCGAAAGGCCGAA ACCAGUU
 463 CUGCAAC CUGAUGAGGCCGAAAGGCCGAA AACCAGU
 466 UGGCTUG CUGAUGAGGCCGAAAGGCCGAA ACAAAACC
 479 CCUCCAA CUGAUGAGGCCGAAAGGCCGAA ADCTUUUG
 480 UCCUCCA CUGAUGAGGCCGAAAGGCCGAA AAUCUUU
 481 CUCCUCC CUGAUGAGGCCGAAAGGCCGAA AAACUCU
 497 GCAGUAA CUGAUGAGGCCGAAAGGCCGAA AUGUCCU
 498 UGCAGUA CUGAUGAGGCCGAAAGGCCGAA ADGUCC
 499 CGCGAG CUGAUGAGGCCGAAAGGCCGAA AAADGUC
 500 ACUGCAG CUGAUGAGGCCGAAAGGCCGAA AAAADGU
 531 AAGGCCU CUGAUGAGGCCGAAAGGCCGAA ACUCUUU
 538 GAAAAAU CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
 539 UGAAAAAU CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 542 UAUUGAA CUGAUGAGGCCGAAAGGCCGAA AUUAAGG
 543 AUAUUGA CUGAUGAGGCCGAAAGGCCGAA AAUUAAG
 544 UAUUJUG CUGAUGAGGCCGAAAGGCCGAA AAAUUA
 545 UUAAUUA CUGAUGAGGCCGAAAGGCCGAA AAAUUA
 549 UAAAUA CUGAUGAGGCCGAAAGGCCGAA AUUGAAA
 551 GUUAAAU CUGAUGAGGCCGAAAGGCCGAA AUAUUGA

554 GAAGUUA CUGADGAGGCCGAAAGGCCGAA AUUAUAU
 555 UGAAGUU CUGADGAGGCCGAAAGGCCGAA AUUAUAU
 556 CGAAGGU CUGADGAGGCCGAAAGGCCGAA AAAAUAU
 560 CCCUCUG CUGADGAGGCCGAAAGGCCGAA AGUAAA
 561 UCCUCU CUGADGAGGCCGAAAGGCCGAA AAGUAAA
 573 AAAAUAU CUGADGAGGCCGAAAGGCCGAA ACUUUCC
 577 CCUGAAA CUGADGAGGCCGAAAGGCCGAA AUUACU
 579 UGCCUGA CUGADGAGGCCGAAAGGCCGAA AUUJUUA
 580 AUGCCUG CUGADGAGGCCGAAAGGCCGAA AAIAUJU
 581 UAUGCCU CUGADGAGGCCGAAAGGCCGAA AAAAUAU
 588 GUGUCAG CUGADGAGGCCGAAAGGCCGAA AUGCCUG
 597 UCUGGCA CUGAUGAGGCCGAAAGGCCGAA AGUGUCA
 598 UUCUGGC CUGAUGAGGCCGAAAGGCCGAA AAGUGUC
 611 AGAAJUU CUGADGAGGCCGAAAGGCCGAA AUGCUUU
 616 UUUUAAG CUGAUGAGGCCGAAAGGCCGAA AUUUUAU
 617 AUUUUAU CUGADGAGGCCGAAAGGCCGAA AAJUUUA
 619 AAJUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAJUU
 620 UAIJUUU CUGAUGAGGCCGAAAGGCCGAA AAGAAUU
 625 UGAAAUU CUGADGAGGCCGAAAGGCCGAA AUUUUAU
 627 UCUGAAA CUGADGAGGCCGAAAGGCCGAA AAIAJUUU
 629 UAUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUUAIJU
 630 AAJADCG CUGADGAGGCCGAAAGGCCGAA AAIAUJU
 631 GAAUUCU CUGAUGAGGCCGAAAGGCCGAA AAIAUUA
 636 AUUCUGA CUGAUGAGGCCGAAAGGCCGAA AAUCUGAA
 638 UGAUUCU CUGAUGAGGCCGAAAGGCCGAA AAJADCG
 644 CUUCAU CUGAUGAGGCCGAAAGGCCGAA AJUCUGA
 647 AUACTUC CUGAUGAGGCCGAAAGGCCGAA AUGAUUC
 653 AGGAAAAA CUGAUGAGGCCGAAAGGCCGAA ACUUCAA
 655 GGAGGAA CUGAUGAGGCCGAAAGGCCGAA AAJACUC
 656 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUACUU
 657 CUUGGAGG CUGADGAGGCCGAAAGGCCGAA AAUAIACU
 658 CCUGGAG CUGAUGAGGCCGAAAGGCCGAA AAAAATAC
 661 UUGCCUG CUGADGAGGCCGAAAGGCCGAA AGGAAAAA
 672 GUAIJAU CUGAUGAGGCCGAAAGGCCGAA AUUUUGC
 676 AAAAGUA CUGAUGAGGCCGAAAGGCCGAA ADCAAUU
 678 AAAAAG CUGAUGAGGCCGAAAGGCCGAA AAJADCAA
 681 AAGAAAAA CUGAUGAGGCCGAAAGGCCGAA AGUAIJAU
 682 UAAGAAA CUGAUGAGGCCGAAAGGCCGAA AAGUAIU
 683 AIAAGAA CUGAUGAGGCCGAAAGGCCGAA AAAGUAIU
 684 AAIAAGA CUGAUGAGGCCGAAAGGCCGAA AAAAGUA
 685 AAAAAG CUGAUGAGGCCGAAAGGCCGAA AAAAAGU
 686 UAAAATA CUGAUGAGGCCGAAAGGCCGAA AAAAAGAG
 688 GUAAAAT CUGAUGAGGCCGAAAGGCCGAA AGAAAAAA
 689 AGUAAA CUGAUGAGGCCGAAAGGCCGAA AAGAAAAA
 691 UAAGUUA CUGAUGAGGCCGAAAGGCCGAA AUAAGAA
 692 UUAAGUU CUGAUGAGGCCGAAAGGCCGAA AAUAAGA
 693 GUUAAGU CUGAUGAGGCCGAAAGGCCGAA AAAUAG
 697 GAAUGUU CUGADGAGGCCGAAAGGCCGAA AGUAAA
 698 AGAUGU CUGAUGAGGCCGAAAGGCCGAA AAGUAAA

703	UUUACAG CUGAUGAGGCCGAAAGGCCGAA AUGUUA
704	UUUACA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
708	GACAUUU CUGAUGAGGCCGAAAGGCCGAA ACAGAAU
715	GUUAACA CUGAUGAGGCCGAAAGGCCGAA ACAIUUU
719	UUAAGUU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
720	AUUAAGU CUGAUGAGGCCGAAAGGCCGAA AACAGAC
724	UACAUUU CUGADGAGGCCGAAAGGCCGAA AGUUAAC
725	AUACAUU CUGADGAGGCCGAAAGGCCGAA AAGUUA
728	UAAAAC CUGADGAGGCCGAAAGGCCGAA AUUAAGU
731	UCAUAAA CUGADGAGGCCGAAAGGCCGAA ACUAUUA
733	UUUCAUU CUGADGAGGCCGAAAGGCCGAA AUACAUU
734	AUUCAUU CUGADGAGGCCGAAAGGCCGAA AATACAU
735	CAUUCUA CUGADGAGGCCGAAAGGCCGAA AAUACU
745	AAUUCUU CUGADGAGGCCGAAAGGCCGAA ACCAUUU
746	AAAUCUU CUGADGAGGCCGAAAGGCCGAA ACCAUU
752	UUUACCA CUGADGAGGCCGAAAGGCCGAA AUUCUUA
753	AUUUACC CUGADGAGGCCGAAAGGCCGAA AAUUCUU
757	ACUAUUU CUGADGAGGCCGAAAGGCCGAA ACCAAAU
761	AAAUACU CUGADGAGGCCGAAAGGCCGAA AUUUACC
762	UAAAAC CUGADGAGGCCGAAAGGCCGAA AAUUAAC
765	AAAUAAA CUGADGAGGCCGAAAGGCCGAA ACUAAUU
767	UUAAAUA CUGADGAGGCCGAAAGGCCGAA AUACUAA
768	AUAAAAU CUGADGAGGCCGAAAGGCCGAA AATACUA
769	CAUAAA CUGADGAGGCCGAAAGGCCGAA AAUACU
771	AAACAUU CUGADGAGGCCGAAAGGCCGAA AAAAAAU
772	UAAACAU CUGADGAGGCCGAAAGGCCGAA AAUAAA
773	AUAAACAU CUGADGAGGCCGAAAGGCCGAA AAUAAA
778	ACAAACAU CUGADGAGGCCGAAAGGCCGAA ACAUUA
779	CACAAAC CUGADGAGGCCGAAAGGCCGAA AACAUUA
783	AGAACAC CUGADGAGGCCGAAAGGCCGAA ACUAAAC
788	UUAUUAG CUGADGAGGCCGAAAGGCCGAA ACACAAAC
789	UUUAUUA CUGADGAGGCCGAAAGGCCGAA AACACAA
791	GUUUUUA CUGADGAGGCCGAAAGGCCGAA AGAACAC
794	UUUGUUU CUGADGAGGCCGAAAGGCCGAA AUUAGAA
805	AGUUGUC CUGADGAGGCCGAAAGGCCGAA AUUUUUG

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	CGCUUU c CUUUGCU	253	AGGGgcU A GaCuuAC
11	uCJUccU U UGCUgAA	259	UaGCAAU a CUGaAgA
12	CJUccUU U GCugAAG	269	GaAGAaU C AAAUCGU
36	GAagacU U CAGAGUC	269	GaAGAaU c AAaCugU
36	GeAgAcU u cAgAGUC	269	GAAGaAU c aAaCugU
37	AAGacUU C AGAGUCA	287	uGGGGGU A CUGUGGA
43	UcaGaGU c AUGAgaA	301	AAAUGCU A UUCcAAA
58	GGAUCCU U CGCACu	301	AAAUGCU a UUCcAAA
59	GAUUCUU C UGCAcUU	303	AUGCUAU u CCaAaAc
59	gADGcUU c UGCAcUU	303	AugCUAU U CcAAAAC
66	CGCAcU U GAGUgUU	304	UgCUAU C cAAAACC
82	UgAcucU c agcUGUG	315	AAcCUGU C aUUAUA
91	GcUgUGU c ugGGOCA	318	cUGUCAU U AUAUAAG
112	ugGAGAU U CCCAuugA	319	UGUCAUU A AUAAGA
113	gGAGAUU C CCAuugAG	322	CaUUAUU A AAGAAAU
141	GAGACCU U GaCACAcG	330	AAGAAAU A CAUUGAC
141	GAgACCU U GaCACAcG	334	AAUACAU U GACCGCC
158	gUCcgCU C AcCGAgC	334	AAUaCaU u GACCgCC
167	ccGAgCU C UGUUGAC	384	AggCAGU U CCUGGAu
196	UGAGGcU U CCUGUCC	385	ggCAGUU C CUGGAuU
197	GAGGCUU C CGUccc	393	CGGGAuU A CCUGCAA
197	gAGGCUU c CGUccc	405	CAAGAGU U CCUUGGU
202	UOCCUGU c CCUacuC	406	AAGAGUU c CUUGGUG
202	UOCCUGU c CcUAcuc	409	AGUuccU U GGUGUgA
206	UGUCccU a cuCaUAA	481	UcacAAU u UAAgUUA
212	UACUCAU a aAAaUCA	482	CAcAAUO U AAgsUUA
212	UacUCAU A AAAAUCA	483	ACAUUUU A AgUUaAa
218	UaaAaaU c accAGCU	483	AcaaAUU a agUUAAa
218	UAAAAAU C ACCAgCU	495	AAAUUgU c AACAgAU
218	uAAAAAU c accAGCU	553	GTUGuuU c CaUUAU
232	uaUGCAU U GGAGAAA	557	UuUccAU U UauaUUU
241	gAGAAA U UUUCAGG	564	UUauAUU u aUgUCCU
241	gAgAAAU c UUUCAGG	564	UUauAUU u AugUCCU
241	gagAAA U UUUCAGG	565	uaUAUUU a ugUCCuG
241	gAgAAAU c UUUCAGG	565	UaUAUUU a UgUCCuG
243	gaaaUcU U UCAGGGg	569	UUUAUGU c cUGUaGU
243	GAAAUCU U UCAGGGg	569	UUAUgGU c cUGUaGU
244	AAAUCUU U CAGGGgc	613	AAAGuGU u uaaccUU
245	AAUCUUU C AGGGgcU	714	AAgUGuU u aAccUUU

620	UUAACcU u uÜUGAU	1407	CCAgUUU A CUcCAGg
793	caAGgCU u UGUcAU	1407	ccAgUUU a CUCCAGG
816	CGGagUU a UACUCcc	1410	GUUUacU C CAGGaaAA
818	GAGUUAU a cUCCcuC	1434	AUGCUUU U aÜUaAU
825	ACUcCcU c CccCUCA	1434	aUgcUUU U AUUAAA
825	acUcccU c CcCcUca	1434	aUgcUUU u AuUAAA
839	AuCcucU U cGUUGCA	1435	UgCUUUU a UuUaAUU
840	uCcucUU c GUUGCAu	1435	ugcUUUU a uUUAaUU
863	cAAGUAU U cCAGGCU	1438	UuUUAUU U AAuUcug
864	AAgUAUJU c CAGGCug	1438	uUUUAUJU U AAuUcug
864	AAgUAUJU c caggCug	1439	UUUAUJU A AUucUgU
913	gAACUCA U GGucCaG	1443	UUUaAUU c UGuaAGa
917	UcÜuggU c CAGGUUGG	1447	ADUCUGU A AGAUGUu
957	UUagcAU c CUUUCeUc	1458	ugUUcaU a UUAAA
960	GCAuccU u UcUcCuA	1458	ugUUcaU A UUAAA
960	GcaUccU u ucUccUa	1460	UcAUAU u AUUAAA
962	AUccUuU c UccUaGC	1461	UcAUauU A UUAAA
975	gccccUU u AgAAUAgA	1463	AUUAUauU U UAUAGa
987	aGAUGAU A cuuAAUG	1475	AUGgAUU c aGUAAgU
990	UGAUACU u AAUgacU	1479	AUUcaGU A AgUUAaU
1000	UGACCUU c UugCuGA	1483	aGuAAGU u AAUAAA
1027	CgggGGCU U ccUgCUC	1483	aGUAAgU U AaUAAA
1034	UCCUGcU C CuUcUaA	1484	GUAAgUU A aUAAA
1037	UgeUCCeU A UcUAAcU	1487	agUUAAA a UUuAUUA
1039	cUccUAU c UAACUUC	1487	AgUUAAU A UUAAA
1039	cUccUAU c UAACUUC	1489	UUAAAaU U UaUAcA
1041	CcUAAcU A ACUUcAA	1489	UUAAuAU u UAUuAcA
1051	UUcAAuU U AAuAccC	1489	UUuAUU U UAUuAcA
1148	uGAcUUU u cUuaUGU	1490	UAAAuUU u AAuAcAc
1213	GCUGGcU u UGGaaa	1490	UAAuAUU U AAuAcAc
1213	geUGGAU u uUgGAAA	1490	UAAuAUU U AAuAcAc
1214	cuGGAUJU U UGGaaaA	1491	AAUADUU a UuaCACg
1215	ugGAUJJU U GGaaaAG	1491	AAUADuU a UuAcAcg
1234	ggGACAU c UccUDDC	1491	AAUADUU A UuAcAcG
1236	GACAUcU c cuUGCAG	1491	AAUADUU A UUAcAcG
1275	ugGGCCU U AcUUCDC	1494	AUuUAUU a CAcgUAU
1276	ggGOCUU A cuUcUCC	1502	CAcGUaU A UaaAUU
1280	CUUAcUU c UCCgUgU	1502	CAcgUAU a UAAUaUU
1298	UgAACUU a AGAaGcA	1507	AUAAUaU a UUcUaaU
1310	gCAAAGU a aUuAccA	1509	AUAAUaU U CUaAuAA
1310	GCAAAGU a aUuAccA	1509	aUaaUaU U CUAAUAA
1310	GcAAAGU a AAAAccA	1510	UAAuAUU C UaAuAAa
1350	AAAGCAU A AAAUggU	1510	UAAuAUU C UaauAAA
1358	AAAUggU U ggGAUgU	1510	UAAuAUU C UaaUAAA
1370	UgUuaUU C AGGUaUC	1510	UaaUaUU C UAAUAAA
1375	UUCAGgU A UCAGggU	1512	aUaUUCU A AUAAAAGC
1377	CAGgUAU C AGggUCA	1515	UUCUAAU A AAgCAGA
1383	UCAGggU C AcUGgAG		
1405	cccCAGU U UACUcCA		

Table 14: Human IL-6 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UACGCCUA AGAA GUCCA ACCAGGGAACCAACGUCCGUCAUUACCUGUA	UGGACCU GGC UGGCCUA
151	GCGTGCAGA AGAA GUCCA ACCAGGGAACCAACGUCCGUCAUUACCUGUA	UCCGACU GCU UCCURUC
172	UCCUUAUC AGAA GAGUC ACCAGGGAACCAACGUCCGUCAUUACCUGUA	GAGAUU GCU GAAGGCA
203	UGUUCUG AGAA GGAAUC ACCAGGGAACCAACGUCCGUCAUUACCUGUA	GAUCCU GUU CCUCUCA

Table 16: Mouse IL-6 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	ACCUAGGA AGAA GACGAC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	GUGUCU GAC UCUUCU
83	CCAGAACAC AGAA GCGAGU ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUCAU GCU GUCCUCG
147	GACGGAC AGAA GUGUCA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UGACACA GGU GUCCUC
150	GGUGAGG AGAA GCUUUG ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	CAUCGU GUC OGCUUCC
154	CCUUGUG AGAA GCPAGC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCG GGU CACGGCC
168	UCCUGUC AGAA GACGUC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCU GGU GACAGCA
179	UGAGUUG AGAA GCGAGC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCU GGU GACAGCA
274	CCOCACG AGAA GUUUA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCU GUC OCUCUCA
381	AUCCAGG AGAA GCGUG ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UCAUACU GUC OGUGGGG
454	CACCAUG AGAA GCUUG ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	CGGGCAU GUU OCUCGAU
499	GUUUUC AGAA GGUUC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	CUGACU GCU OCUCGGG
548	UAAAUGC AGAA GCAAU ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	GUGACCA GUU GCAAAC
701	CCAGGGG AGAA GAAAU ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	AUUCGU GUU UCUCUUA
710	GAAGAGGA AGAA GCGGCA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCU GGU OCUCUCG
870	AGLUCAA AGAA GCGUG ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	CGGGCU GAC UUCAACU
919	CUCGCCC AGAA GGACCA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UCCUCA GGU GGACCGAG
1030	UGAAGGG AGAA GGAGGC ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	ACCUUCU GCU OCUCUUA
1170	AUGGCCA AGAA GUUCA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UGAUAUCA GAC UCUUCAU
1205	CAAAUCC AGAA GCUCA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UGGAGCA GCU GGAUUUG
1402	CUGGAGA AGAA GGGGA ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	UCCOCCA GGU UACUCG
1421	AGCCAUAC AGAA GUUUU ACCAGGAAACACACCCUUCUCCUCAUACCUUGA	AAAACA GAU GUACGU

Table 16 : Mouse IL-5 Hairpin Ribozyme Sequences

Position	Hairpin Ribozyme Sequence	Substrate
75	ACUUGGA AGAA GAAAC ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	GUUCUU GUC UCUCAGGU
89	CCGCGAC AGAA GAGGU ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	ACCUCA GCU GUUCUAG
147	GCGGCC AGAA GUGCA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UGCAUA GCU GUUCUAG
150	CCUCCAG AGAA GCUUU ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	CGCCAU GUC UCUCUAC
154	AGAA GAAAC ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	GUUCGC GCU CGGGAGC
168	UCCUAG AGAA GPGGC ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	GGCCU GU GAGAGCA
199	UGAGUAG AGAA GGAGG ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	GUUCUU GUC GUUCUAG
274	CCCCAG AGAA GUUGA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UCAAACU GUC UCUCUAG
381	ANUCAGG AGAA GCGCG ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	CGCGCA GUU OCUCUAG
454	CAACUUG AGAA GCGCG ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	CUGGUU GCU CCUCUAG
499	GUUUUC AGAA GGUUC ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	GUCACCA GCU GAAAAC
548	UAAAUGG AGAA GCUAU ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	AUUGGU GUU UCACAUUA
701	GGCAAGGG AGAA GGAUU ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	AAUUCU GUU CCUCUAG
710	GAAGGAGG AGAA GGGCA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UCCUCCU GUC UCUCUAC
870	ACUUCAAA AGAA GCGGG ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	OCGGGU GAC UUUCACU
919	CUGCGUC AGAA GGACCA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UCGUCA GAU CGGGCGC
1030	UCCGUCCG AGAA GGAGC ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	CCUUCU GCU CCUCUAC
1170	AUCGCCCA AGAA GAUCA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UGAUUA GCU UCUCUACU
1205	CAAAUCC AGAA GCUCA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UGGCGCA GCU GCUUUC
1402	CUGGCGTA AGAA GGGGA ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	UCCOCA GGU UUUCACG
1421	AAGCAUC AGAA GUUUU ACCGGGAAACCAAGGUACGUUCGUACAUACCUA	AAAMCA GAU GUUCUU

Table 17

Mouse *re/A* HH Target sequence

nt.	Position	HH Target Sequence	nt.	Position	HH Target Sequence
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19		AAUGGCCU a caCaGgA	467		CCAGGCCU c cuGuUCg
22		aGCUCCu a CGUggGUG	469		AaGCaAU u AGeCAGC
26		CcUCcaU u GcGgACa	473		UuUgAGU C AGauCAG
93		GauCUGU U uCCCCUc	481		AGCGeAU C CAGACCA
94		AuUCUGUU U CCCUCA	501		AACCCCCU U uCACGUU
100		UuCCCCU C ADUOUC	502		ACCCCCU U CACGUU
103		CCCUCAU C UUUCCCU	508		UuCACGU U CCTADAG
105		CUCADCU U uCCCuCA	509		uCACGUU C CTuAAGA
106		UCADCUU U CCCuCAG	512		cGUUCCU A TAGAgGA
129		CAGGCUU C UGGgCCU	514		UCCCUAU A GAGGGAGC
138		GGgCCUU A DGUGGAG	534		GGGGACU A uGACuUG
148		UGGAGAU C ADGGAAc	556		UGGGCCU C TGCUUCC
151		AGAUCAU c GAGCAGC	561		CUCOGCU U CCAGGUG
180		ADGOGAU U CGGCUAU	562		UCOGCUU C CAGGUGA
181		UGCGAUU C CGGCUAU	585		aAGCCAU U AGccAGc
186		UUCOGCU A uAAAUGC	598		GGCCCCU C CUCCUGA
204		GGGOGCU C aGCGGGC	613		CCCCUGU C CTCeuCAC
217		GCAGUAU U CCuGGCG	616		CUGUCCU C uCACAUc
239		CAcAGAU A CCACCA	617		guCCCUU C CUCAGCC
262		CCACCAU C AAGAUCA	620		CCUUCGU C AgCCaUg
268		UCAAGAU C AAUGGCU	623		UCCUGeU U CCADUCUc
276		AAUGGCU A CACAGGA	628		AUCCGau U UUUGAUa
301		UuCGaAU C UCCCUGG	630		CCsAUuuU U UGAuAAC
303		CGaAUcU C CCUGGUC	631		CgAUuUU U GAuAACC
310		CCUUGGU C ACCAAGG	638		UGgcCAU U GGGuuCC
323		GGccccU C CUCCuga	661		CCGAGCU C AAGAUcU
326		UCCaccU C ACCGGCC	667		UCAAGAU C UGGCGAG
335		CCGGCCU C AUCCACA	687		CGGAACU C UGGgAGC
349		AuGAaAU U GUgGGgA	700		GCUGCCU C GGUGGGG
352		AGAucaU c GaAcAGc	715		AUGAGAU C UUCuUgC
375		GAUggCU a CTADGAG	717		GACAUcU U CuUgCUG
376		AUGGucU C UccGgaG	718		AGAUcUU C uUgCUGU
378		GGCUAcU A UGAGGCCU	721		UueUCCU c CaUUGeG
391		CGAGCU C UGCCCCaG	751		AaGACAU U GAGGUGU
409		GCAGUAU C CAuAGEU	759		GAGGUGU A UUUCACG
416		CCgCAGU a UCCuAAG	761		GGUGUAU U UCAcGGG
417		CAuAGEU U CCAGAAC	762		GUGUAUU U CACGGGA
418		AuAGcUU C CAGAAC	763		UGUAUUU C ACggGAC
433		UGGGgAU C CAGUGUG	792		CGAGGCCU C CTUUUcU
795		GGCUCCU U UUUCCAA	1167		GAUGAGU U UUCCeCC
796		GCUCUUU U UUCAAG	1168		AUGAGGU U UCCeCCA
797		CUCCUUU U CuCAAGC	1169		UGAGUUU U CCeCCAU
798		UCCUUUU C uCAAGCU	1182		AUGcUGU U aCCaUCA
829		UGGCCAU U GUGuUCC	1183		UGcUGUU a CCaUCAg

834	AUUGUGU U CGGGACU	1184	GGccccU C CUccUGa
835	UUGUGUU C CGGAUCU	1187	GUccCUU C CUcaGCC
845	GACUCCU C CgUACGC	1188	UUacCAU C aGGGCAG
849	CCUCCgU A CGCCGAC	1198	GGgAGUU u AGuCuGa
872	CCAGGCU C CUGUuCG	1209	CAGCCCCU a caCCUUC
883	UuCGaGU C UCCADGC	1215	cuGGCCU U aGCaCCG
885	CGaGUU C CAUGCAG	1229	GGuCCCU u CCucAGc
905	GGGGCCU U CUGAUuCG	1237	CCCAgeU C CUGCCCC
906	CGGGCJU C uGAuCGC	1250	CCAGCCU C CAGgGUc
919	GcGAGCU C AGUGAGC	1268	CCCAGCU C CuGCCCC
936	AUGGAGU U CCAGUAC	1279	CCAUUGGU C ccuuCCu
937	UGGA <u>g</u> UU C CAGUACU	1281	gUGGgeU C AGCuGeG
942	UUCAGU A CuUGCCA	1286	AUgAGUu U UccCCCA
953	GCCuCAU c CACAuGA	1309	CUCCUGU u CgAGUcu
962	AGAuGAU C GcCACCG	1315	ccccAGU u CTAaACC
963	CagUacU u gCCaGAc	1318	CAGUUCU A aCCCCggG
973	ACCGGU U GAGAGA	1331	gGGUCCU C CcCAGUC
986	GAGACeU u cMAgagu	1334	CuuUUCU C AaGCTUGa
996	AGGACCU A UGAGACC	1389	AOGCUGU C gGAaGCC
1005	GAGACCU U CAGAGU	1413	CUGCAGU U UGAUGcU
1006	AGACCUU C AAGAGUA	1414	UGCAGUU U GAUGcUG
1015	AGAGUAU C ADGAAGA	1437	GGGGCCU U CCTUUGGC
1028	GAAGAGU C CUUUCAA	1441	CCUUGCU U GGCAAACA
1031	GAGUCCU U UCAuUGG	1467	GgaGUGU U CACAGAC
1032	AGUCCUU U CAAuGGA	1468	gAGUGUU C ACAGACC
1033	GGCCUUU C AaUGGAC	1482	CUGGCAU C uGUgGAC
1058	COGGCCU C CAAccCG	1486	CuUCggU a GggAACU
1064	UaCACCU U GAuCCAA	1494	GACAAU C aGAGUUU
1072	GgCGUAU U GCUGUGC	1500	UCAgAGU U UCAGCAG
1082	UGUGCCU a COOGAAA	1501	CaGAGUU U CAGCAGC
1083	aaGOCUU C CGGAAGU	1502	aGAGUUU C AGCAGCU
1092	CGAAACU C AACUCCU	1525	gGuGCAU C CCTUGUGu
1097	CCAAACU U CUGUOOC	1566	AUGGAGU A CCCUGAA
1098	UCAACUU C UGUOOCC	1577	UGAaGCU A UAAUCUG
1102	CUUCUGU C CCCAACG	1579	AaGCTAU A ACUCGCC
1125	CAGCCCU A cacCUUC	1583	UATAACU C GCCUggU
1127	GGCAUUAU a gCcUUAC	1588	CUUCUCU A GaGAgGG
1131	cAUCCCU c agCacCA	1622	CCCAGCU C CUGCCCC
1132	AcacCUU C ccAGCAU	1628	UCCUGGU U CggUaGG
1133	UCCAUcU c CagCuUC	1648	GGGGGU U CCCAAUg
1137	UUUACQU U AgCgCgc	1660	cUGACCU C ugcccAG
1140	cCAGCAU C CCTcAGC	1663	cuCUGGU U CCAGGUg
1153	GCACCAU C AACUuUG	1664	uCuGGUU C CAGGUGA
1158	ADCAACU U UGAUGAG	1665	CUCgeUU U CGGAGgU
1680	GAAGACU U CUCCUCC		
1681	AAGACU U C UCCUCCA		
1683	GACJUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U CGGGACA		

1704	AUGGACU U CUCUGCU
1705	UGGACUU C UCUGCUC
1707	GACUUCU C UGGUUCU
1721	UUGAGU C AGAUCAg
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGU
1734	AGCUCCU A AGGUGeU
1754	CaGugCU C CCaAGAG

Table 18
 Human *rel A* HH Target Sequences
 nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AUUGGU C GUUCGUA	467	GCGGCCU A UCAGUCA
22	GGCUUGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCAAG	473	UACAGU C AGCGCAU
93	GAACUGU U CCCCCUC	481	AGCGCAU C CAGACCA
94	AACUGUU C CCCCCUA	501	AACCCU U CCAAGUU
100	UCCCCCU C AUCCUCC	502	ACCCCU C CAAGUCC
103	CCUCAU C UUCCCCG	508	UCCAAU U CCUATAG
105	CUCACU U CCCCCGA	509	CCAAGUU C CUUAGA
106	UCACUCCU C CCGGCAG	512	AGUCCU A UAGAAGA
129	CAGGCCU C UGGCCCC	514	UCCUAU A AAAGAGC
138	GGCCCCU A UGUGGAG	534	GGGGACU A CGACCTG
148	UGGAGAU C AUUGAGC	556	UGGGCCU C UGCJUCC
151	AGAUCAU U GAGCAGC	561	CUCUGCU U CCAGGUG
180	AUGGCCU U CGCCUAC	562	UCUGCUU C CAGGUGA
181	UGGCCUU C CGCUACA	585	GACCCAU C AGGCAGG
186	UUCGCCU A CAAGUGC	598	GGCCCCU C CGCTUGC
204	GGGCCCU C CGGGGGC	613	CGCCUGU C CUUCCUC
217	GCAGCAU C CGAGGCG	616	CGUCCU U CCTUAC
239	CACAGAU A CCACCAA	617	UGUCCUU C CUCATCC
262	CCACCAU C AAGADCA	620	CCUCCU C AUCCCAU
268	UCAAGAU C AAUGGUU	623	CCUCAU C CCAUCU
276	AAUGGU A CACAGGA	628	AUCCCAU C UUUGACA
301	UGCGCAU C UGCCUGG	630	CCCAUCU U UGACAAU
303	CGCAUCU C CCUGGGC	631	CCAUUU U GACAAUC
310	CCCUGGU C ACCAAGG	638	UGACAAU C GUGCCCC
323	GGACCCU C CUCACCG	661	CCGAGCU C AAGAUU
326	CCCUCU C ACCGGCC	667	UCAAGAU C UGCCGAG
335	CCGGCCU C ACCCCCCA	687	CGAAACU C UGGCAGC
349	ACGAGCU U GTAGGAA	700	GTUGCCU C GGUGGGG
352	AGCUUGU A GGAAAGG	715	AUGAGAU C UUCCUAC
375	GAUGGUU U CTADGAG	717	GAGAUU U CCUACUG
376	AUGGUUU C UADGAGG	718	AGAUUU C CUACUGU
378	GGCUUCU A UGAGGU	721	UCTUCCU A CUGUGUG
391	CGAGCU C UGCCGGG	751	AGGACAU U GAGGUGU
409	GCTUGCAU C CACAGUU	759	GAGGUGU A UUUCACG
416	CCACAGU U UCCAGAA	761	GGUGUAU U UCACGGG
417	CACAGUU U CCAGAAC	762	GGUGUAU U CACGGGA
418	ACAGUUU C CAGAAC	763	UGUAUUU C ACGGGAC
433	UGGGAAU C CAGUGUG	792	CGAGGUU C CUUUCUG
795	GGCUUCU U UUOGCAA	1167	GAUGAGU U UCCCACC
796	GCTUCCUU U UCGCAAG	1168	AUGAGUU U CCCACCA
797	CUCCUUU U CGCAAGC	1169	UGAGGUU C CCACCAU
798	UCCUUUU C GCAAGCU	1182	AUGGUUU U UCCUUCU
829	UGGCCAU U GUGUUCC	1183	UGGUUU U CCTUUCUG
834	AUUGUGU U CGGGACC	1184	GGUGUUU C CUUCUGG

835	UUGUGUU C CGGACCC	1187	GUUUCCU U CUUGGCAG
845	GACCCCU C CCTAACG	1188	UUUCCUU C UGGGCAG
849	CCUCCCCU A CGCAGAC	1198	GGCAGAU C AGCCAGG
872	GCAGGCCU C CGUGGCG	1209	CAGGCCU C GGCCUUG
883	UGCGGUGU C UCCAUGC	1215	UCCGGCCU U GGCCCCG
885	CGUGUCU C CAUGCA	1229	GGCCCCU C CCCAAGU
905	GCGGCCU U CCTACCG	1237	CCCAGAU C CUGCCCC
906	CGGCCUU C CGACCCG	1250	CCAGGCCU C CAGCCCC
919	GGGAGCU C AGUGAGC	1268	CCCUGCU C CAGCCAU
936	AUGGAAU U CCAGUAC	1279	CCAGGGU A UCAGCUC
937	UGGAAUU C CAGUACC	1281	ADGGUAU C AGCUCUG
942	UCCAGU A CCTGCCA	1286	ADCAGCU C UGGCCCA
953	GCCAGAU A CAGACGA	1309	CCCCUGU C CCAGUCC
962	AGACGAU C GUACCG	1315	UCCAGU C CUAGCCC
965	CGAUCGU C ACAGGAU	1318	CAGUCU A GCCCCAG
973	ACCGGAU U GAGGAGA	1331	AGGCCCCU C CUCAGGC
986	GAAACGU A AAAGGAC	1334	CCCCUCU C AGGGUGU
996	AGGACAU A UGAGACC	1389	ACCGUGU C AGAGGCC
1005	GAGACCU U CAAAGAC	1413	CUGCAGU U UGAGAU
1006	AGACCUU C AAGAGCA	1414	UGCAGUU U GAUGAUG
1015	AGAGCAU C ADGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUUCAG	1441	CCUUGCU U GGCACAC
1031	GAGUCU U UCAAGGG	1467	GCUGUGU U CACAGAC
1032	AGUCCUU U CAGGGAA	1468	CUGUGUU C ACAGACC
1033	GUCCUUU C AGGGGAC	1482	CUGGCAU C CGUCGAC
1058	CGGGCCU C CAUCUCC	1486	CAUCCGU C GACAACU
1064	UCCACCU C GACGCAU	1494	GACAACU C CGAGUUU
1072	GACGCAU U GCUGUGC	1500	UCCGAGU U UCAGCAG
1082	UGUGCCU U CCGCGAG	1501	COGAGUU U CAGCAGC
1083	GUGCCUU C CGCGAGC	1502	CGAGUUU C AGCAGCU
1092	CGCAGCU C AGCUUCU	1525	AGGGCAU A CCTUGUGG
1097	CUCAGCU U CUGUCCC	1566	ADGGAGU A CCCUGAG
1098	UCAGCUU C UGUCCCC	1577	UGAGGCU A UUACUCG
1102	CUUCUGU C CCCAGC	1579	AGGCUAU A ACTUOGCC
1125	CAGCCCCU A UCCCCUU	1583	UAAAACU C GCCUAGU
1127	GCCCCAU C CCTTUCAC	1588	CUCGCCU A GUGACAG
1131	UAUCCCCU U UACGUCA	1622	CCCAGCU C CUGCUCC
1132	AUCOCUU U ACCUCAU	1628	UCCUGCU C CACUGGG
1133	UCCCCUU A CGUCAUC	1648	CGGGGCCU C CCCAUG
1137	UUUACGU C AUCCCCG	1660	AUGGCCU C CUUUCAG
1140	ACGUCAU C CCUGAGC	1663	GCCUCCU U UCAGGAG
1153	GCACCAU C AACUAGG	1664	CCUCCUU U CAGGAGA
1158	AUCAACU A UGAUGAG	1665	CUCCUUU C AGGAGAU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GGGGACA		
1704	AUGGACU U CUCAGCC		

1705	UGGACUU C UCAGCCC
1707	GACCUUCU C AGCCCCUG
1721	GCGAGGU C AGAUCAG
1726	GUCAGAU C AGCUUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUUCCU A AGGGGGU
1754	CUGCCCCU C CCCAGAG

Table 19
Mouse *rel A* HH Ribozyme Sequences
nt.
Sequence

	HH Ribozyme Sequence
19	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACCAAG CUGAUGAGGCCGAAAGGCCGAA AGGAGCT
26	UGUCCGC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
93	GAGGGGA CUGAUGAGGCCGAAAGGCCGAA ACAGADU
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGAU
100	GAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGAA
103	AGGGAAA CUGAUGAGGCCGAAAGGCCGAA AUAGAGG
105	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGAGGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	AGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAGCCUG
138	CUCACAC CUGAUGAGGCCGAAAGGCCGAA AAGGCC
148	GUUCGAU CUGAUGAGGCCGAAAGGCCGAA AUUCUCA
151	GCUUUC CUGAUGAGGCCGAAAGGCCGAA ADGADCU
180	AUAGCGG CUGAUGAGGCCGAAAGGCCGAA AUUCGAU
181	UAUAGCG CUGAUGAGGCCGAAAGGCCGAA AAUCGCA
186	GCAUUUA CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
204	GCCCGCU CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	CGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUUCUG
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCUUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGG CUGAUGAGGCCGAAAGGCCGAA AUUCGAA
303	GRACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUUCG
310	CCUJUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGA
335	UGUGGAA CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	UCCCCAC CUGAUGAGGCCGAAAGGCCGAA AGUUCAU
352	GCTGUUC CUGAUGAGGCCGAAAGGCCGAA ADGAUCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCADC
376	CUCCGGA CUGAUGAGGCCGAAAGGCCGAA AGAACAU
378	AGOCUCA CUGAUGAGGCCGAAAGGCCGAA AGUAGCC
391	CUGGGCA CUGAUGAGGCCGAAAGGCCGAA AGGUUCAG
409	AGCTUAG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
416	CTAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUGCGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AGCTUAG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCTUAU
433	CACACUG CUGAUGAGGCCGAAAGGCCGAA AUCCCCA
467	CGAACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
469	GCUUGGU CUGAUGAGGCCGAAAGGCCGAA AUUGCUU
473	CUGAUUC CUGAUGAGGCCGAAAGGCCGAA ACUAAA
481	UGGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU

501 AACGUGA CUGAUGAGGCCGAAAGGCCGAA AGGGGUU
 502 GAACGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGGU
 508 CTUATAGG CUGAUGAGGCCGAAAGGCCGAA ACGUGAA
 509 UCTAUAG CUGAUGAGGCCGAAAGGCCGAA AACGUGA
 512 UCCUCUA CUGAUGAGGCCGAAAGGCCGAA AGGAACG
 514 GCUCCUC CUGAUGAGGCCGAAAGGCCGAA ATAGGAA
 534 CAAGUCA CUGAUGAGGCCGAAAGGCCGAA AGUCCCC
 556 GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGGGGCA
 561 CACCUUG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
 562 UCACCUUG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
 585 GCUGGCU CUGAUGAGGCCGAAAGGCCGAA ADGGCUU
 598 UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
 613 GUGAGAG CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
 616 GADGUGA CUGAUGAGGCCGAAAGGCCGAA AGGACAG
 617 GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
 620 CADGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
 623 GAGADGG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
 628 UACAAA CUGAUGAGGCCGAAAGGCCGAA ADGGGAI
 630 GUUACCA CUGAUGAGGCCGAAAGGCCGAA AAADCGG
 631 GGUACAC CUGAUGAGGCCGAAAGGCCGAA AAADCG
 638 GGAACAC CUGAUGAGGCCGAAAGGCCGAA AUUGCCA
 661 AGAUUU CUGAUGAGGCCGAAAGGCCGAA AGCUCCG
 667 CUCGGCA CUGAUGAGGCCGAAAGGCCGAA ADGUUGA
 687 GCUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUUCCG
 700 CCCCACCC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 715 GCAAGAA CUGAUGAGGCCGAAAGGCCGAA AUUCUCAU
 717 CAGCAAG CUGAUGAGGCCGAAAGGCCGAA AGAUUCU
 718 ACAGCAA CUGAUGAGGCCGAAAGGCCGAA AAGAUU
 721 CGCAAUG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
 751 ACACCUC CUGAUGAGGCCGAAAGGCCGAA AUUGUUU
 759 CGUGAAA CUGAUGAGGCCGAAAGGCCGAA ACACCUC
 761 CCGUGUGA CUGAUGAGGCCGAAAGGCCGAA AUACACC
 762 UCCCGUG CUGAUGAGGCCGAAAGGCCGAA AAATACAC
 763 GCCCCGU CUGAUGAGGCCGAAAGGCCGAA AAATACA
 792 AGAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCCTUOG
 795 UUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGAGCC
 796 CUUGAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAGC
 797 GCUUGAG CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
 798 AGGUUGA CUGAUGAGGCCGAAAGGCCGAA AAAAGGA
 829 GGAACAC CUGAUGAGGCCGAAAGGCCGAA AUUGCCA
 834 AGUUCGG CUGAUGAGGCCGAAAGGCCGAA ACACAAU
 835 GAGUUCG CUGAUGAGGCCGAAAGGCCGAA AACACAA
 845 CGGUACG CUGAUGAGGCCGAAAGGCCGAA AGGAGUC
 849 GUOSGGG CUGAUGAGGCCGAAAGGCCGAA ACGGAGG
 872 CGAACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUUG
 883 GCAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCGAA
 885 CGUCAG CUGAUGAGGCCGAAAGGCCGAA AGACUCG
 905 CGAUUCAG CUGAUGAGGCCGAAAGGCCGAA AGGCCGC
 906 CGGAUCA CUGAUGAGGCCGAAAGGCCGAA AAGGCCG

919 GCUCACU CUGAUGAGGCCGAAAGCCGAA AGCUCCG
 936 GUACUGG CUGAUGAGGCCGAAAGCCGAA ACUCCAU
 937 AGUACUG CUGAUGAGGCCGAAAGCCGAA AACTCCA
 942 UGGCAAG CUGAUGAGGCCGAAAGCCGAA ACUGGAA
 953 UCADUGG CUGAUGAGGCCGAAAGCCGAA AUGAGGC
 962 CGGUGGC CUGAUGAGGCCGAAAGCCGAA AUCAUCU
 965 GUCUGGC CUGAUGAGGCCGAAAGCCGAA AGUACUG
 973 UCUCUUC CUGAUGAGGCCGAAAGCCGAA AUCCGGU
 986 ACUCUUG CUGAUGAGGCCGAAAGCCGAA AGGUUC
 996 GGUCUCA CUGAUGAGGCCGAAAGCCGAA AGGUCCU
 1005 ACUCUUG CUGAUGAGGCCGAAAGCCGAA AGGUUC
 1006 UACUCUU CUGAUGAGGCCGAAAGCCGAA AAGGUUC
 1015 UCUUCAU CUGAUGAGGCCGAAAGCCGAA AUACUCU
 1028 UUGAAAG CUGAUGAGGCCGAAAGCCGAA ACUCUUC
 1031 CCATUGA CUGAUGAGGCCGAAAGCCGAA AGGACUC
 1032 UCCAUUG CUGAUGAGGCCGAAAGCCGAA AAGGACU
 1033 GUCCAUU CUGAUGAGGCCGAAAGCCGAA AAAGGAC
 1058 CGGGUUG CUGAUGAGGCCGAAAGCCGAA AGGCCGG
 1064 UUGGAUC CUGAUGAGGCCGAAAGCCGAA AGGUUGA
 1072 CCACAGC CUGAUGAGGCCGAAAGCCGAA AUACGCC
 1082 UUUCCCC CUGAUGAGGCCGAAAGCCGAA AGGCACA
 1083 ACUUCGG CUGAUGAGGCCGAAAGCCGAA AAGGCUU
 1092 AGAAGUU CUGAUGAGGCCGAAAGCCGAA AGUUUCG
 1097 GGGACAG CUGAUGAGGCCGAAAGCCGAA AGUUGAG
 1098 GGGGACA CUGAUGAGGCCGAAAGCCGAA AAGUUGA
 1102 CCUUGGG CUGAUGAGGCCGAAAGCCGAA ACAGAAG
 1125 GAAGGUG CUGAUGAGGCCGAAAGCCGAA AGGGCUG
 1127 GUAAGGC CUGAUGAGGCCGAAAGCCGAA AUADGGC
 1131 UGGUGCU CUGAUGAGGCCGAAAGCCGAA AGGGAG
 1132 AUUGCUG CUGAUGAGGCCGAAAGCCGAA AAGGUGU
 1133 GAAGCUG CUGAUGAGGCCGAAAGCCGAA AGADGGA
 1137 GCGCGCU CUGAUGAGGCCGAAAGCCGAA AAGUAAA
 1140 GCUGAGG CUGAUGAGGCCGAAAGCCGAA AUUGCUG
 1153 CAAAGUU CUGAUGAGGCCGAAAGCCGAA AUGGUGC
 1158 CUCACUA CUGAUGAGGCCGAAAGCCGAA AGUUGAU
 1167 GGGGGAA CUGAUGAGGCCGAAAGCCGAA ACUCAUC
 1168 UGGGGGA CUGAUGAGGCCGAAAGCCGAA AACUCAU
 1169 AUGGGGG CUGAUGAGGCCGAAAGCCGAA AAACUCA
 1182 UGAUGGU CUGAUGAGGCCGAAAGCCGAA ACAGCAU
 1183 CUGAUGG CUGAUGAGGCCGAAAGCCGAA AACAGCA
 1184 UCAGGGG CUGAUGAGGCCGAAAGCCGAA AGGGGCC
 1187 GGCUGAG CUGAUGAGGCCGAAAGCCGAA AAGGGAC
 1188 CGGCCCU CUGAUGAGGCCGAAAGCCGAA AUGGUAA
 1198 UCAGACU CUGAUGAGGCCGAAAGCCGAA AACUCCC
 1209 GAAGGUG CUGAUGAGGCCGAAAGCCGAA AGGGCUG
 1215 CGGUGCU CUGAUGAGGCCGAAAGCCGAA AGGCCAG
 1229 CCTUGAGG CUGAUGAGGCCGAAAGCCGAA AGGGACC
 1237 GGGGCAG CUGAUGAGGCCGAAAGCCGAA AGCUGGG
 1250 GAGCCUG CUGAUGAGGCCGAAAGCCGAA AGGCUGG

1268 GGGGCAG CUGAUGAGGCGGAAAGGCCGAA AGCTGGG
 1279 AGGAAGG CUGAUGAGGCGGAAAGGCCGAA ACCAUGG
 1281 CGCAGCU CUGAUGAGGCGGAAAGGCCGAA AGCCCAC
 1286 UGGGGGA CUGAUGAGGCGGAAAGGCCGAA AACUCAU
 1309 AGACUCC CUGAUGAGGCGGAAAGGCCGAA ACAGGAG
 1315 GGGUUAU CUGAUGAGGCGGAAAGGCCGAA ACUGGGG
 1318 CGGGGGU CUGAUGAGGCGGAAAGGCCGAA AGAACUG
 1331 GACUGGG CUGAUGAGGCGGAAAGGCCGAA AGGAACC
 1334 UCAGCUU CUGAUGAGGCGGAAAGGCCGAA AGAAAAG
 1389 GGCCTUCC CUGAUGAGGCGGAAAGGCCGAA ACAGCGU
 1413 AGCAUCA CUGAUGAGGCGGAAAGGCCGAA ACTGCGG
 1414 CAGCAUC CUGAUGAGGCGGAAAGGCCGAA AACUGCA
 1437 GCGAAGC CUGAUGAGGCGGAAAGGCCGAA AGGCCCC
 1441 UGUUGCC CUGAUGAGGCGGAAAGGCCGAA AGCAAGG
 1467 GUCUGUG CUGAUGAGGCGGAAAGGCCGAA ACACUCC
 1468 GGUUUGU CUGAUGAGGCGGAAAGGCCGAA AACACUC
 1482 GUCCACA CUGAUGAGGCGGAAAGGCCGAA AUGCCAG
 1486 AGUUCCC CUGAUGAGGCGGAAAGGCCGAA ACGGAAG
 1494 AAACUCA CUGAUGAGGCGGAAAGGCCGAA AGUUGUC
 1500 CUGCUUA CUGAUGAGGCGGAAAGGCCGAA ACUCUGA
 1501 GCUGCUG CUGAUGAGGCGGAAAGGCCGAA AACUCUG
 1502 AGCUGCU CUGAUGAGGCGGAAAGGCCGAA AAACUCU
 1525 ACACAGG CUGAUGAGGCGGAAAGGCCGAA AUGCACC
 1566 UUCAGGG CUGAUGAGGCGGAAAGGCCGAA ACUCCAU
 1577 CGAGUUA CUGAUGAGGCGGAAAGGCCGAA AGCUUCA
 1579 GGCGAGU CUGAUGAGGCGGAAAGGCCGAA AUAGCUU
 1583 ACCAGGC CUGAUGAGGCGGAAAGGCCGAA AGUUAUA
 1588 CCCUCUC CUGAUGAGGCGGAAAGGCCGAA AGGAGAG
 1622 GGGGCAG CUGAUGAGGCGGAAAGGCCGAA AGCTGGG
 1628 CCTAACCG CUGAUGAGGCGGAAAGGCCGAA AGCAGGA
 1648 CAUUGGG CUGAUGAGGCGGAAAGGCCGAA AGCCCCG
 1660 CGGGGCA CUGAUGAGGCGGAAAGGCCGAA AGGUCAG
 1663 CACCUUG CUGAUGAGGCGGAAAGGCCGAA AGCAGAG
 1664 UCACCUU CUGAUGAGGCGGAAAGGCCGAA AAGCAGA
 1665 ACCUUCG CUGAUGAGGCGGAAAGGCCGAA AAGCGAG
 1680 GGAGGAG CUGAUGAGGCGGAAAGGCCGAA AGCUUUC
 1681 UGGAGGA CUGAUGAGGCGGAAAGGCCGAA AAGUCUU
 1683 AAUUGGAG CUGAUGAGGCGGAAAGGCCGAA AGAAGUC
 1686 CGCAAUG CUGAUGAGGCGGAAAGGCCGAA AGGAGAA
 1690 UGUCCCG CUGAUGAGGCGGAAAGGCCGAA AUUGGAGG
 1704 AGCAGAG CUGAUGAGGCGGAAAGGCCGAA AGUCCAU
 1705 GAGCAGA CUGAUGAGGCGGAAAGGCCGAA AAGUCCA
 1707 AAGAGCA CUGAUGAGGCGGAAAGGCCGAA AGAAGUC
 1721 CGAUCU CUGAUGAGGCGGAAAGGCCGAA ACUAAA
 1726 AGGAGCU CUGAUGAGGCGGAAAGGCCGAA AUUCUGAC
 1731 ACCUUAG CUGAUGAGGCGGAAAGGCCGAA AGCTUGAU
 1734 AGCACCU CUGAUGAGGCGGAAAGGCCGAA AGGAGCU
 1754 CUUUGG CUGAUGAGGCGGAAAGGCCGAA AGCACUG

Table 20

Human *rel A* HH Ribozyme Sequences

nt. Position

HH Ribozyme Sequences

19	UACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACTACA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
26	CGUGCAC CUGAUGAGGCCGAAAGGCCGAA ACAGACG
93	GAGGGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUUU
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGUU
100	GGAGAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGGG
103	CGGGGAA CUGAUGAGGCCGAAAGGCCGAA AUAGAGG
105	UGCCCGG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGCCGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	GGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
138	CUCCACA CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
148	GTUCAAU CUGAUGAGGCCGAAAGGCCGAA AUUCUCA
151	GCUGUCU CUGAUGAGGCCGAAAGGCCGAA AUAGADCU
180	GUAGCGG CUGAUGAGGCCGAAAGGCCGAA AGCGCAU
181	UGUAGCG CUGAUGAGGCCGAAAGGCCGAA AAGCGCA
186	GCACUUG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
204	GGGGCGG CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	OGCUGG CUGAUGAGGCCGAAAGGCCGAA AUUGCUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUUCUGU
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUJUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGG CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
303	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
310	CCUJUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
335	UGGGGGU CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	JUCCUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCGU
352	CCUUUCC CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCAUJ
376	CCUCAUJ CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
378	AGCCUCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
391	COGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
409	AACUGUG CUGAUGAGGCCGAAAGGCCGAA AUJGAGC
416	JUUCGGA CUGAUGAGGCCGAAAGGCCGAA ACUGUGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AACUGUG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAACUGU
433	CACACTUG CUGAUGAGGCCGAAAGGCCGAA AUUCCCCA
467	UGACTUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGC
469	GTUGACU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
473	AUGCGCU CUGAUGAGGCCGAAAGGCCGAA ACUGAUA
481	UGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
501	AACTUJGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUU

502 GACUUG CUGAUGAGGCCGAAAGGCGAA AAGGGGU
 508 CUAUAGG CUGAUGAGGCCGAAAGGCGAA ACUJUGGA
 509 UCUAUAG CUGAUGAGGCCGAAAGGCGAA AACUJUGG
 512 UCUUCUA CUGAUGAGGCCGAAAGGCGAA AGGAACU
 514 GCUCUUC CUGAUGAGGCCGAAAGGCGAA ATAGGAA
 534 CAGGUUC CUGAUGAGGCCGAAAGGCGAA AGUCCCC
 556 GGAAGCA CUGAUGAGGCCGAAAGGCGAA AGCCGCA
 561 CACCTGG CUGAUGAGGCCGAAAGGCGAA AGCAGAG
 562 UCACCTG CUGAUGAGGCCGAAAGGCGAA AAGCAGA
 585 CCUGCCU CUGAUGAGGCCGAAAGGCGAA ADGGGUC
 598 GCAGGCG CUGAUGAGGCCGAAAGGCGAA AGGGGCC
 613 GAGGAGG CUGAUGAGGCCGAAAGGCGAA ACAGGGG
 616 GAUGAGG CUGAUGAGGCCGAAAGGCGAA AGGACAG
 617 GGADGAG CUGAUGAGGCCGAAAGGCGAA AAGGACA
 620 AUGGGAU CUGAUGAGGCCGAAAGGCGAA AGGAAGG
 623 AAGADGG CUGAUGAGGCCGAAAGGCGAA ADGAGGA
 628 UGUAAA CUGAUGAGGCCGAAAGGCGAA ADGGGAAU
 630 AUUGUCA CUGAUGAGGCCGAAAGGCGAA AGADGGG
 631 GAUUGUC CUGAUGAGGCCGAAAGGCGAA AAGAUGG
 638 GGGGCAC CUGAUGAGGCCGAAAGGCGAA AUUGUCA
 661 AGAUCUU CUGAUGAGGCCGAAAGGCGAA AGCUUCG
 667 CUOGGCA CUGAUGAGGCCGAAAGGCGAA ADCUUGA
 687 GCUGCCA CUGAUGAGGCCGAAAGGCGAA AGUUUCG
 700 CCCACCC CUGAUGAGGCCGAAAGGCGAA AGGCAGC
 715 GUAGGAA CUGAUGAGGCCGAAAGGCGAA ADUCUAI
 717 CAGUAGG CUGAUGAGGCCGAAAGGCGAA AGAUCUC
 718 ACAGUAG CUGAUGAGGCCGAAAGGCGAA AAGAUCU
 721 CACACAG CUGAUGAGGCCGAAAGGCGAA AGGAAGA
 751 ACACCUC CUGAUGAGGCCGAAAGGCGAA ADGUCCU
 759 CGUGAAA CUGAUGAGGCCGAAAGGCGAA ACACCU
 761 CCCUGUA CUGAUGAGGCCGAAAGGCGAA AUACACC
 762 UCCUGUG CUGAUGAGGCCGAAAGGCGAA AUACAC
 763 GUCCCGU CUGAUGAGGCCGAAAGGCGAA AAATACA
 792 CGAAAAG CUGAUGAGGCCGAAAGGCGAA AGCCUCG
 795 UUGCGAA CUGAUGAGGCCGAAAGGCGAA AGGAGCC
 796 CUUGCGA CUGAUGAGGCCGAAAGGCGAA AAGGAGC
 797 CCUUGCG CUGAUGAGGCCGAAAGGCGAA AAAGGAG
 798 AGCUUGC CUGAUGAGGCCGAAAGGCGAA AAAAGGA
 829 GGAACAC CUGAUGAGGCCGAAAGGCGAA ADGGCCA
 834 GGUCCGG CUGAUGAGGCCGAAAGGCGAA ACACAAU
 835 GGGUCCG CUGAUGAGGCCGAAAGGCGAA AACACAA
 845 GGGUAGG CUGAUGAGGCCGAAAGGCGAA AGGGGUC
 849 GUCUGCG CUGAUGAGGCCGAAAGGCGAA AGGGAGG
 872 CGCACAG CUGAUGAGGCCGAAAGGCGAA AGOCUGC
 883 GCPUGGA CUGAUGAGGCCGAAAGGCGAA ACAOGCA
 885 CUGCAAG CUGAUGAGGCCGAAAGGCGAA AGACACG
 905 CGGUCCG CUGAUGAGGCCGAAAGGCGAA AGGOOGC
 906 CCGGUUC CUGAUGAGGCCGAAAGGCGAA AAGGOOG
 919 GCUCACU CUGAUGAGGCCGAAAGGCGAA AGCUCCC

936	GUACUGG CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
937	GGUACUG CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
942	UGGCAGG CUGAUGAGGCCGAAAGGCCGAA ACUGGAA
953	UCGUCUG CUGAUGAGGCCGAAAGGCCGAA ADUGGCC
962	CGGUGAC CUGAUGAGGCCGAAAGGCCGAA ADUGUCU
965	AUCGGGU CUGAUGAGGCCGAAAGGCCGAA ACGAUUC
973	UCUCCUC CUGAUGAGGCCGAAAGGCCGAA ADCCGGU
986	GUCCUUU CUGAUGAGGCCGAAAGGCCGAA ACGUUUC
996	GGUCUCA CUGAUGAGGCCGAAAGGCCGAA ADGUCCU
1005	GCUCUUG CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
1006	UGCUCUU CUGAUGAGGCCGAAAGGCCGAA AAGGUCCU
1015	UCUUCAU CUGAUGAGGCCGAAAGGCCGAA ADGCUCCJ
1028	CTGAAAG CUGAUGAGGCCGAAAGGCCGAA ACUCCUC
1031	CCGUGA CUGAUGAGGCCGAAAGGCCGAA AGGACUC
1032	UCCGUG CUGAUGAGGCCGAAAGGCCGAA AAGGACU
1033	GUCCGU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
1058	CGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
1064	ADGCGUC CUGAUGAGGCCGAAAGGCCGAA AGGUUGGA
1072	GCACAGC CUGAUGAGGCCGAAAGGCCGAA ADGCGUC
1082	CUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGGCACA
1083	GCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCAC
1092	AGAACUU CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
1097	GGGACAG CUGAUGAGGCCGAAAGGCCGAA AGGUUGAG
1098	GGGGACA CUGAUGAGGCCGAAAGGCCGAA AAGGUUA
1102	GCUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
1125	AAAGGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
1127	GUAAAAG CUGAUGAGGCCGAAAGGCCGAA AUAGGGC
1131	UGACGUA CUGAUGAGGCCGAAAGGCCGAA AGGGATA
1132	AUGACGU CUGAUGAGGCCGAAAGGCCGAA AAGGGAU
1133	GAUGACG CUGAUGAGGCCGAAAGGCCGAA AAAGGGG
1137	CAGGGAU CUGAUGAGGCCGAAAGGCCGAA ACCUAAA
1140	GCUCAGG CUGAUGAGGCCGAAAGGCCGAA ADGACGU
1153	CAUAGUU CUGAUGAGGCCGAAAGGCCGAA ADGGUGC
1158	CUCAUCA CUGAUGAGGCCGAAAGGCCGAA AGGUUAU
1167	GGUGGGG CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
1168	UGGUGGG CUGAUGAGGCCGAAAGGCCGAA AACUCAU
1169	ADGGUGG CUGAUGAGGCCGAAAGGCCGAA AAACUCA
1182	AGAAGGA CUGAUGAGGCCGAAAGGCCGAA ACACCAU
1183	CGAGAAG CUGAUGAGGCCGAAAGGCCGAA AACACCA
1184	CCAGAAG CUGAUGAGGCCGAAAGGCCGAA AAACACC
1187	UGCCCCAG CUGAUGAGGCCGAAAGGCCGAA AGGAAAC
1188	CUGCCCC CUGAUGAGGCCGAAAGGCCGAA AAGGAAA
1198	CCUCCGU CUGAUGAGGCCGAAAGGCCGAA ADUGGCC
1209	CAAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
1215	CGGGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
1229	ACUUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
1237	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
1250	GGGGCUG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
1268	AUGGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGG

1279	GAGCUGA CUGAUGAGGCGAAAGGCCGAA ACCAUGG
1281	CAGAGCU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
1286	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1309	GGACUGG CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
1315	GGGCTAG CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
1318	CUGGGGC CUGAUGAGGCCGAAAGGCCGAA AGGACUG
1331	GCCUGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCTU
1334	ACAGCCU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
1389	GGCCUCU CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
1413	AUCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
1414	CAUCADC CUGAUGAGGCCGAAAGGCCGAA AACUGCA
1437	GCCAAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
1441	UGUUGGC CUGAUGAGGCCGAAAGGCCGAA AGCAAGG
1467	GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACACAGC
1468	GGUCUGU CUGAUGAGGCCGAAAGGCCGAA AACACAG
1482	GUCCACG CUGAUGAGGCCGAAAGGCCGAA ADGCCAG
1486	AGUUGUC CUGAUGAGGCCGAAAGGCCGAA ACGGAUG
1494	AAACUUG CUGAUGAGGCCGAAAGGCCGAA AGUUGUC
1500	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACTUOGGA
1501	GCUGCUG CUGAUGAGGCCGAAAGGCCGAA AACUOGG
1502	AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AAACUOG
1525	CCACAGG CUGAUGAGGCCGAAAGGCCGAA ADGCCUU
1566	CUCAGGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
1577	CGAGUUA CUGAUGAGGCCGAAAGGCCGAA AGCCUCA
1579	GGCGAGU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
1583	ACTAGGC CUGAUGAGGCCGAAAGGCCGAA AGUUAUA
1588	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AGGGCAG
1622	GGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCUOGG
1628	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1648	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
1660	CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
1663	CUCCUGA CUGAUGAGGCCGAAAGGCCGAA AGGAGGC
1664	UCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGGAGG
1665	AUCUCCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
1680	GGAGGAG CUGAUGAGGCCGAAAGGCCGAA AGUCUUC
1681	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAGUCUU
1683	AAUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1686	CGCAAUG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
1690	UGUOOGC CUGAUGAGGCCGAAAGGCCGAA AUUGGAGG
1704	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCAU
1705	GGCCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCA
1707	CAAGGGCU CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1721	CUGACU CUGAUGAGGCCGAAAGGCCGAA ACUCAGC
1726	AGGACCU CUGAUGAGGCCGAAAGGCCGAA AUUCGAC
1731	CCCTUAG CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1734	ACCCUCU CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
1754	CUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCAG

Table 21
Human *relA* Hairpin Ribozyme/Target Sequences
Hairpin Ribozyme sequence

nt. Position	Substrate
90	ucagggggg agaa guuc accaggaaaacacacguugugguaacauuaccggua
156	ccuccuug agaa gcuc accaggaaaacacacguugugguaacauuaccggua
362	ccccauccc agaa guc accaggaaaacacacguugugguaacauuaccggua
413	guucugga agaa gugg accaggaaaacacacguugugguaacauuaccggua
606	gaaaaaca agaa gcmc accaggaaaacacacguugugguaacauuaccggua
652	uucggcc agaa gugu accaggaaaacacacguugugguaacauuaccggua
695	cccaccga agaa ccug accaggaaaacacacguugugguaacauuaccggua
853	agccucgg agaa cggu accaggaaaacacacguugugguaacauuaccggua
900	ccuccggm agaa gccc accaggaaaacacacguugugguaacauuaccggua
955	ugacgaucc agaa gurw accaggaaaacacacguugugguaacauuaccggua
1037	guccggg agaa ggub accaggaaaacacacguugugguaacauuaccggua
1045	ggccccgg agaa gugo accaggaaaacacacguugugguaacauuaccggua
1410	caucauca agaa gcau accaggaaaacacacguugugguaacauuaccggua
1453	acaggcgg agaa gugc accaggaaaacacacguugugguaacauuaccggua
1471	gaucggcg agaa guga accaggaaaacacacguugugguaacauuaccggua

Table 22
Mouse *rel A* Hairpin Ribozyme/Target Sequences
Hairpin Ribozyme sequence

nt. Position	Substrate
137	GUUGCUUC AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
273	GAGAUUCG AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
343	GCCAUCCC AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
366	GGGCAGAG AGAA GCUU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
633	UUGGCCUC AGAA GUGU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
676	CCCACCGA AGAA GCUU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
834	AGGUGGG AGAA GCGU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
881	GAUCAGAA AGAA GCGG ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1100	AGGUGUAG AGAA GCGG ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1205	GGGCAGAG AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1361	GGGCUUC AGAA GCGU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1385	CAGCAUCA AGAA GCGG ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1431	ACUCUCCG AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1449	GAUGGCCG AGAA GUUA ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
1802	AAGUCGGG AGAA GCUU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
2009	UGGCUCCA AGAA GUUC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
2124	UGGUGUCC AGAA GCGC ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
2233	AUUCGAAA AGAA GCGA ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA
2354	UCAGUAAA AGAA GCUU ACCAGAGAAACACCCUUGGGGUACAUUACUGGUAA

Table 23: Human TNF- α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GGCAGGU U CUCUUC	321	GJCAGAU C AUCCUCU
29	GCAGGUU C UCUCUCC	324	AGAUCAU C UUCUCGA
31	A GGUCUCU C UUCUCU	326	AUCAUCAU U CCUGAAC
33	GUUCUCU U CCUCUCA	327	UCAUCUU C UCGAACCC
34	UUCUCUU C CUCUCAC	329	AUCUUCU C GAACCCC
37	UCUUCUU C UCACATA	352	AGCCUGU A GCGGAG
39	UUCUCU C ACACAU	361	CCCAUGU U GUAGCAA
44	CCACAU A CGACCC	364	AUGUUGU A GCRAACC
58	CAACGGCU C CAACUC	374	AAACCCU C AAGCUGA
65	CCACCCU C UCUCCCC	391	GGCAGGU C CAGGGC
67	ACCCUCU C UCCUCUG	421	AUGGCCU C CUGGCGA
69	CCUCUCU C CCCUGGA	449	GAGAGAU A ACCAGCU
106	GCAGAU C CGGGACG	468	GUGCCAU C AGAGGGC
136	AGGCGCU C COCAAGA	480	GGCCUGU A CCTCAUC
165	CAGGGCU C CAGGGGG	484	UGUACCU C AUCUACU
177	CGGUGCU U GUUUCUC	487	ACCUCAU C UACUCCC
180	UGCUGUGU U CCUCAGC	489	CTCAUCU A CUCCAG
181	GCTTGGUU C CUCAGCC	492	AUCUACU C CCAGGUC
184	UGUUCUU C AGCCUCU	499	CCACAGU C CUCUUC
190	UCAAGCU C UUCUCUC	502	AGGUCCU C UUCAAGG
192	AGACUCU U CUCUUC	504	GUCCUCU U CAAGGGC
193	GCCTCUCU C UCCUUC	505	UCCUCUU C AAGGGCC
195	CUCUCUCU C CUUCUC	525	UCCCCCU C CACCAU
198	UUCUCUCU U CCTGAGC	538	AUGUGCU C CUACACC
199	UCUCCUU C CGAGUCG	541	UGCUCU C ACCCACA
205	UCCUGAU C GUCCAG	553	ACACCAU C AGCGCA
226	CCACGUU C UUCUGCC	562	GGGGCAU C GGGGCU
228	ACGCUCU U CGGCCUG	568	UCCGGGU C UCCUACC
229	CGCUCUU C UGCGUGC	570	GGGGGUU C CUACCG
243	CUGCACU U UGGAGUG	573	GUCCUCU A CCAGACC
244	UGCACUU U GGAGUGA	586	CCAAAGGU C AACCUCC
253	GAGUGAU C GGCCCCC	592	UCAACCU C CUCUCUG
273	GAAGAGU C CCCCAGG	595	ACCUCCU C UCUGCCA
286	GGGACCU C UCUCUAA	597	GUCCUCU C UGCCAUC
288	GACCUUCU C UCUAADC	604	UCCGGGU C AAGAGCC
290	CCUCUCU C UAAUCAG	657	CCCUGGU A UGAGCCC
292	UCUCUCU A AUCAGCC	667	AGCCCCAU C UAUCUGG
295	CUCUAAU C AGCCCCU	669	CCCAUCU A UCGGGGA
302	CGGCCCCU C UGGCGCA		

671	CAUCUAU C UGGGAGG	960	UGGGAUU C AGGAUAG
682	GAGGGGU C UGCCAGC	1001	AACCACU A AGAAUUC
684	GGGGUCU U CCAGCTG	1007	UAGAAAU U CAACUGG
685	GGGUCUU C CAGCTGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGCGCUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AADGGC	1029	CAGAACU C ACUGGGG
725	GAUCAAU C GGCGCGA	1040	GGGGCCU A CAGCTUU
735	CCCGACU A UCUCGAC	1046	UACAGCU U UGAUCCC
737	CGACTAU C UCGACUU	1047	ACAGCTU U GAUCCCU
739	ACUACCU C GACUUUG	1051	CUUUGAU C CCUGACA
744	CCCGACU U UGCGAG	1060	CGACAU C UGGAUIC
745	UCCACUU U GOOGAGU	1067	CTGGAAU C UGGAGAC
753	GGCGAGU C UGGGCAG	1085	GGAGCCU U UGGJUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCUG
765	CAGGUCU A CUUUGGG	1090	CUUUGGU U CGGGCCA
768	GUCAUCU U UGGGAUC	1091	UUUGGUU C UGGCCAG
769	UCUACUU U GGGGAUCA	1113	CAGGACU U GAGAAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGACACU U GOOCUGU	1129	CUACAU A GAAADUG
801	CGAACAU C CAAOCUU	1135	UAGRAAU U GACCAA
808	CCAAACCU U COCAAAC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAAACG	1152	GGACCTU A GGCCTUC
820	AACGCCU C COCTGCC	1158	UAGGCACU U CCUCUCU
833	CCCCAAU C CCTUUUAU	1159	AGGCCUU C CUCUCUC
837	AACCCCU U UAUUACC	1162	CCUUCU C UCUCAG
838	AUCCCUU U AUUACCC	1164	UUCUCU C UCCAGAU
839	UCCCUUU A UUACCCC	1166	CCUCUCU C CAGAUGJ
841	CCUUUAU U ACCCCCCU	1174	CAGAUTU U UCCAGAC
842	CUUUAU A CCCCCUC	1175	AGAUGUU U CCAGACU
849	ACCCCCU C CUUCAGA	1176	GAUGJUU C CAGACUU
852	CCUCCU U CAGACAC	1183	CCAGACU U CCUGAG
853	CCUCCUU C AGACACC	1184	CAGACUU C CUUGAGA
863	ACACCCU C AACCCU	1187	ACUUCCU U GAGACAC
869	UCAACCU C UUCUGGC	1208	CAGCCCU C CCCAUGG
871	AACCUCU U CUCCUC	1224	GCCAGCU C CCUCUAU
872	ACCUCUU C UGGCUCA	1228	GUUCCCCU C UAUUUAU
878	UCUGGUU C AAAAGAG	1230	UCCUCU A UUUAUGU
890	AGAGAAU U GGGGGCU	1232	CCUCUAU U UADGUUU
898	GGGGGCU U AGGGJUCG	1233	CUCUAUU U AUGUUUG
899	GGGGCUU A GGGUCGG	1234	UCUAUUU A UGUUUGC
904	UUGGGGU C GGAACCC	1238	UUUAUGU U UGCACUU
917	CCAGCU U AGAACUU	1239	UUADGUU U GCACUUG
918	CAAGCUU A GAACUU	1245	UUGCACU U GJGAUUA
924	UAGAACU U UAGCAA	1251	UUGUGAU U AUUUAUU
925	AGAACUU U AAGCAAC	1252	UGUGAUU A UUUAUUA
926	GAACUU A AGCAACA	1254	UGAUUAU U UAUUAUU
945	CACCAUC U CGAAACC	1255	GAUUAU U AUUUAUU
946	ACCACUU C GAAACCU	1256	AUUAUUA A UUUAUUA
959	CUUGGUU U CAGGAU	1258	UAUUAU U AUUUAUU

1259	AUUAUUA A UUUAGUU	1440	UGUUUUU U AAAAUAU
1261	UUAAUUA U UAUUUAU	1441	GUUUUUU A AAUAUUA
1262	UAUUAU U AUUUAU	1446	UUAUAU A UUAUCUG
1263	AUUAUUA A UUUAUUA	1448	AAAUAU U ACCUGAU
1265	UAUUAU U UAUUAU	1449	AAUAUUA A UCCGAUU
1266	AUUAUUA U AUUAUUA	1451	AUUAUUA C UGAUUA
1267	UUUAUUA A UUUAUUA	1456	AUCUGAU U AAGJUGU
1269	UAUUAU U AUUUAU	1457	UCUGAU A AGJUGUC
1270	AUUAUUA A UUUAUUA	1461	AUUAAGU U GGTAAA
1272	UUUAUUA U UAUUAU	1464	AAGGUGU C UAAACAA
1273	UAUUAU U AUUUAU	1466	GUGUGCU A AACAAUG
1274	AUUAUUA A UUUAUUA	1479	UGCUGAU U UGGUGAC
1276	UAUUAU U UAUUAU	1480	GCGGATU U GGTGACC
1277	AUUAUUA U AUUUAU	1494	CAACJGU C ACUCAU
1278	UUUAUUA A UUACAG	1498	UGUCACU C ATGCGUG
1280	UAUUAU U UACAGAU	1501	CACTCAU U GCTGAGG
1281	AUUAUUA U ACAGAUG	1512	GAGGCCU C UGCTCCCC
1282	UUUAUUA A CAGAUGA	1517	CUCUGCU C CCCAGGG
1294	UGAAGGU A UUUAUUA	1528	AGGGAGU U GGGCTTG
1296	AAUGAU U UAUUUGG	1533	GUUGUGU C UGUAUAC
1297	AUGUAU U AUUUGGG	1537	UGUCUGU A AUCCGCC
1298	UGUAUUA A UUUGGG	1540	CUGUAU C GGCTUAC
1300	UAUUAU U UGGGAGA	1546	UCGGCCU A CUATUCA
1301	AUUAUUA U GGGAGAC	1549	GCCUACU A UUCAGCG
1315	CGGGGGU A UCCUGGG	1551	CUACUAU U CAGGGGC
1317	GGGGGTAU C CGGGGGG	1552	UACUAU C AGGGCG
1334	CCAAUGU A GGAGCG	1566	GAGAAA A AAGGJUG
1345	GCUGCCU U GGCUCAG	1572	UAAAGGU U GCTUAGG
1350	CUUGGCCU C AGACATG	1576	GGUUGCU U AGGAAAG
1359	GACADGU U UCCCGUG	1577	GUUGCU A GGAAAGA
1360	ACAUGUU U UCCGUGA		
1361	CAUGUU U CGUGAAA		
1362	ADGUUUU C CGUGAAA		
1386	GAACAAU A GGCTGGU		
1393	AGGCUGU U CCACAGU		
1394	GGCTGGU C CCACAGA		
1401	CCACAGU A GCCCCCU		
1414	CUUGGCCU C UGUGGCCU		
1422	UGUGACU U CUUUGA		
1423	GUGCCUU C UUUGGAU		
1425	GCCUUCU U UGAAUUA		
1426	CCUUCUU U UGAAUUA		
1427	CUUCUUU U GAUUAUG		
1431	UUUUGAU U ADGUUUU		
1432	UUUGAU A UGUUUUU		
1436	AUUAUGU U UUUAAAA		
1437	UUADGU U UUUAAAA		
1438	UAUGUU U UUAAAUA		

Table 24: Human TNF- α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCUGC
31	AGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
33	TGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAC
34	GUGAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
37	UADGUGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
39	AQUAUGU CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
44	GGGUCAAG CUGAUGAGGCCGAAAGGCCGAA AUGUGAG
58	GAAGGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
63	GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
67	CAAGGGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCCGAAAGGCCGAA ADCAUGC
136	UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCCU
165	CCGCCCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCCUG
177	GAGGAAC CUGAUGAGGCCGAAAGGCCGAA AGCAACG
180	GCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AACRAAGC
184	AGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAACA
190	AGGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
192	GAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
193	GGAAAGG CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
199	CGAUCAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA ADCAAGA
226	GGCAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
228	CAAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAGCCU
229	GCAGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
243	CACTUCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAG
244	UCACUCC CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
253	GGGGGCC CUGAUGAGGCCGAAAGGCCGAA ADCAUC
273	CTTUGGGG CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
286	UUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUCCC
288	GAUUTAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
290	CUGAUUA CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
302	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGGCTG

321 AGAAGAU CUGAUGGCCCCGAAAGGCCGAA AUCUGAC
 324 UCGAGAA CUGAUGGCCCCGAAAGGCCGAA AUCGAUC
 326 GUUUCGAG CUGAUGGCCCCGAAAGGCCGAA AGAAGAU
 327 GGUUUCGA CUGAUGGCCCCGAAAGGCCGAA AAGAUGC
 329 GGGGUUC CUGAUGGCCCCGAAAGGCCGAA AGAAGAU
 352 CAUGGGC CUGAUGGCCCCGAAAGGCCGAA ACAGGCC
 361 UUGCUAC CUGAUGGCCCCGAAAGGCCGAA ACAGGG
 364 GGUUUGC CUGAUGGCCCCGAAAGGCCGAA ACAACAU
 374 UCAGCUU CUGAUGGCCCCGAAAGGCCGAA AGGGUU
 391 GGCACUG CUGAUGGCCCCGAAAGGCCGAA AGCUGCC
 421 UGGCCAG CUGAUGGCCCCGAAAGGCCGAA AGGGCAU
 449 AGCTGGU CUGAUGGCCCCGAAAGGCCGAA AUUCUC
 468 GCCCUCU CUGAUGGCCCCGAAAGGCCGAA AUGGCAC
 480 GAAGGAG CUGAUGGCCCCGAAAGGCCGAA ACAGGCC
 484 AGUAGAU CUGAUGGCCCCGAAAGGCCGAA AGGUACA
 487 GGGAGUA CUGAUGGCCCCGAAAGGCCGAA ADGAGGU
 489 CGGGGAG CUGAUGGCCCCGAAAGGCCGAA AGAAGAG
 492 GACCUUG CUGAUGGCCCCGAAAGGCCGAA AGUAGAU
 499 UGAAGAG CUGAUGGCCCCGAAAGGCCGAA ACCUUGG
 502 CCUUGAA CUGAUGGCCCCGAAAGGCCGAA AGGACCU
 504 GCCCUUG CUGAUGGCCCCGAAAGGCCGAA AGAGGAC
 505 GGGCCUU CUGAUGGCCCCGAAAGGCCGAA AAGAGGA
 525 AUGGGUG CUGAUGGCCCCGAAAGGCCGAA AGGGCA
 538 GGGUGAG CUGAUGGCCCCGAAAGGCCGAA AGCACAU
 541 UGUGGGU CUGAUGGCCCCGAAAGGCCGAA AGGAGCA
 553 UGCGGGU CUGAUGGCCCCGAAAGGCCGAA AUGGUGU
 562 AGACGGC CUGAUGGCCCCGAAAGGCCGAA AUGGGGC
 568 GGUAGGA CUGAUGGCCCCGAAAGGCCGAA ACGGCCA
 570 CTGGUAG CUGAUGGCCCCGAAAGGCCGAA AGACGGC
 573 GGUCUGG CUGAUGGCCCCGAAAGGCCGAA AGGAGAC
 586 GGAGGUU CUGAUGGCCCCGAAAGGCCGAA ACCUUGG
 592 CAGAGAG CUGAUGGCCCCGAAAGGCCGAA AGGUUGA
 595 UGGCAGA CUGAUGGCCCCGAAAGGCCGAA AGGAGGU
 597 GAUGGCA CUGAUGGCCCCGAAAGGCCGAA AGAGGAG
 604 GGCUCUU CUGAUGGCCCCGAAAGGCCGAA AUGGCAG
 657 GGGCUCA CUGAUGGCCCCGAAAGGCCGAA ACCIAGGG
 667 CCAGAUU CUGAUGGCCCCGAAAGGCCGAA AUGGGU
 669 UOCCAGA CUGAUGGCCCCGAAAGGCCGAA AGAUAGGG
 671 CCTCCCCA CUGAUGGCCCCGAAAGGCCGAA AUAGAUG
 682 GTUGGAA CUGAUGGCCCCGAAAGGCCGAA ACCCCUC
 684 CAGCUGG CUGAUGGCCCCGAAAGGCCGAA AGACCCC
 685 CCAGCUG CUGAUGGCCCCGAAAGGCCGAA AAGACCC
 709 CAGCGCU CUGAUGGCCCCGAAAGGCCGAA AGUCGGU
 721 GCGGAUU CUGAUGGCCCCGAAAGGCCGAA AUUCUC
 725 UCGGGCC CUGAUGGCCCCGAAAGGCCGAA AUJUGAU
 735 GUCGAGA CUGAUGGCCCCGAAAGGCCGAA AGUCGGG
 737 AAGUCGA CUGAUGGCCCCGAAAGGCCGAA AUAGUCG
 739 CAAAGUC CUGAUGGCCCCGAAAGGCCGAA AGAUAGU
 744 CUCGGCA CUGAUGGCCCCGAAAGGCCGAA AGUCGAG

745 ACUUCGGC CUGAUGAGGCCGAAAGGCCGAA AAGUCGA
 753 CUGCCCA CUGAUGAGGCCGAAAGGCCGAA ACUUCGGC
 763 CAAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
 765 CCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCUJG
 768 GAAUCCA CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 769 UGAUCCC CUGAUGAGGCCGAAAGGCCGAA AGUAGA
 775 GGGCAAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCAA
 778 ACAGGGC CUGAUGAGGCCGAAAGGCCGAA ADGAUCC
 801 AAGGUUG CUGAUGAGGCCGAAAGGCCGAA ADGUUCG
 808 GUUUUGG CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
 809 CGUUUUGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
 820 GGCAGGG CUGAUGAGGCCGAAAGGCCGAA AGGUU
 833 AUAAAAGG CUGAUGAGGCCGAAAGGCCGAA AUUCCCC
 837 GGUAATA CUGAUGAGGCCGAAAGGCCGAA AGGGAUU
 838 GGGUAAU CUGAUGAGGCCGAAAGGCCGAA AAGGGAU
 839 GGGGUAA CUGAUGAGGCCGAAAGGCCGAA AAAGGGA
 841 AGGGGGGU CUGAUGAGGCCGAAAGGCCGAA AUAAAAGG
 842 GAGGGGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAG
 849 UCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGGGGU
 852 GUGUCUG CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
 853 GGUGUCU CUGAUGAGGCCGAAAGGCCGAA AAGGAGG
 863 AGAGGUU CUGAUGAGGCCGAAAGGCCGAA AGGGGU
 869 GCGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
 871 GAGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAGGU
 872 UGAGCCA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
 878 UCUUUUU CUGAUGAGGCCGAAAGGCCGAA AGCCAGA
 890 AGCCCCC CUGAUGAGGCCGAAAGGCCGAA AUUCUCU
 898 CGACCCU CUGAUGAGGCCGAAAGGCCGAA AGCCCCC
 899 CGAACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
 904 GGGUUCC CUGAUGAGGCCGAAAGGCCGAA ACCUUAA
 917 AAUUCUC CUGAUGAGGCCGAAAGGCCGAA AGCUU
 918 AAAGGUUC CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
 924 UUGCCTUA CUGAUGAGGCCGAAAGGCCGAA AGUUCUA
 925 GUUGCUU CUGAUGAGGCCGAAAGGCCGAA AAGUUCU
 926 UGUUGCU CUGAUGAGGCCGAAAGGCCGAA AAAGGUUC
 945 GGUUUCG CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 946 AGGUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUGGU
 959 AUUCCUG CUGAUGAGGCCGAAAGGCCGAA AUUCCAG
 960 CAUCCCCU CUGAUGAGGCCGAAAGGCCGAA AUUCCCA
 1001 GAADUCU CUGAUGAGGCCGAAAGGCCGAA AGUGGGU
 1007 CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
 1008 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCUU
 1021 AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
 1029 CCCCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
 1040 AAAGCUG CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
 1046 GGGAUCA CUGAUGAGGCCGAAAGGCCGAA AGCUGUA
 1047 AGGGAUU CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
 1051 UGUCAGG CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
 1060 GAAUCCA CUGAUGAGGCCGAAAGGCCGAA ADGUACG

1067 GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AUUCCAG
 1085 AGAACCA CUGAUGAGGCCGAAAGGCCGAA AGGCUC
 1086 CAGAACC CUGAUGAGGCCGAAAGGCCGAA AAGGCUC
 1090 UGGCCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAAG
 1091 CGGGCCA CUGAUGAGGCCGAAAGGCCGAA AACCAA
 1113 UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGUCCUG
 1124 UCUAGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCUU
 1129 CAUUCUC CUGAUGAGGCCGAAAGGCCGAA AGGUAGAG
 1135 UUGUGUC CUGAUGAGGCCGAAAGGCCGAA AUUUCUA
 1151 AAGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCA
 1152 GAAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUCC
 1158 AGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCUA
 1159 GAGAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 1162 CGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
 1164 AUUCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
 1166 ACACCTG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
 1174 GUCCUGG CUGAUGAGGCCGAAAGGCCGAA ACACUCG
 1175 AGUCUGG CUGAUGAGGCCGAAAGGCCGAA AACACU
 1176 AAGUCUG CUGAUGAGGCCGAAAGGCCGAA AAACABC
 1183 CUCAAGG CUGAUGAGGCCGAAAGGCCGAA AGUCUGG
 1184 UCUCAGG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
 1187 GUGUCUC CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
 1208 CCAUAGG CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
 1224 ATAGAGG CUGAUGAGGCCGAAAGGCCGAA AGCUCGG
 1228 ATAATAA CUGAUGAGGCCGAAAGGCCGAA AGGGAGC
 1230 ACATAAA CUGAUGAGGCCGAAAGGCCGAA AGAGGGG
 1232 AAACATA CUGAUGAGGCCGAAAGGCCGAA ATAGAGG
 1233 CAAACAU CUGAUGAGGCCGAAAGGCCGAA AAUAGAG
 1234 GCAAACA CUGAUGAGGCCGAAAGGCCGAA AAUATAGA
 1238 AAGUGCA CUGAUGAGGCCGAAAGGCCGAA ACATAAA
 1239 CAAGUGC CUGAUGAGGCCGAAAGGCCGAA AACATAA
 1245 UAAUCAC CUGAUGAGGCCGAAAGGCCGAA AGUGCAA
 1251 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUACCAA
 1252 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUCACA
 1254 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAUCA
 1255 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAUC
 1256 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAU
 1258 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1259 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1261 ATAAATA CUGAUGAGGCCGAAAGGCCGAA ATAAATA
 1262 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAAATA
 1263 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1265 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAAATA
 1266 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAAUAA
 1267 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1269 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAAUAA
 1270 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1272 ATAAATA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA
 1273 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA

1274 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1276 GUAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAA
 1277 UGUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1278 CGUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1280 AUCUGUA CUGAUGAGGCCGAAAGGCCGAA AUAAA
 1281 CAUCUGU CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1282 UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1294 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
 1296 CCAAAA CUGAUGAGGCCGAAAGGCCGAA AUACAU
 1297 CCCAAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
 1298 UCCCAAA CUGAUGAGGCCGAAAGGCCGAA AAUACAC
 1300 UCCCGCA CUGAUGAGGCCGAAAGGCCGAA AUAAA
 1301 GUCCCCC CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1315 CCCAGGA CUGAUGAGGCCGAAAGGCCGAA ACCCGGG
 1317 CCCCGAG CUGAUGAGGCCGAAAGGCCGAA AUACCCC
 1334 CAGCUCC CUGAUGAGGCCGAAAGGCCGAA ACACUUG
 1345 CGAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 1350 CADGUCU CUGAUGAGGCCGAAAGGCCGAA AGCCCAAG
 1359 CACGGAA CUGAUGAGGCCGAAAGGCCGAA ACACUGC
 1360 UCAOGGA CUGAUGAGGCCGAAAGGCCGAA AACADGU
 1361 UUCAACGG CUGAUGAGGCCGAAAGGCCGAA AAACADG
 1362 UUCCACG CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1386 AACAGCC CUGAUGAGGCCGAAAGGCCGAA AUUUGUC
 1393 ACAUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGCCU
 1394 UACADGG CUGAUGAGGCCGAAAGGCCGAA AACAGCC
 1401 AGGGGGC CUGAUGAGGCCGAAAGGCCGAA ACAUGGG
 1414 AGGCACA CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
 1422 UCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGGCACA
 1423 AUCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGGCAC
 1425 UAAUCAA CUGAUGAGGCCGAAAGGCCGAA AGAACCC
 1426 AUAAUCA CUGAUGAGGCCGAAAGGCCGAA AAGAACG
 1427 CAUAAUC CUGAUGAGGCCGAAAGGCCGAA AAAGRAAG
 1431 AAAACAU CUGAUGAGGCCGAAAGGCCGAA AUCAAAA
 1432 AAAAACCA CUGAUGAGGCCGAAAGGCCGAA AUCAAA
 1436 UUUAAAA CUGAUGAGGCCGAAAGGCCGAA ACACUAA
 1437 UUUUAAA CUGAUGAGGCCGAAAGGCCGAA AACACAA
 1438 AUUUUAA CUGAUGAGGCCGAAAGGCCGAA AACACAA
 1439 UAUUUUA CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1440 AUAUUUU CUGAUGAGGCCGAAAGGCCGAA AAAAACCA
 1441 AAUAUUU CUGAUGAGGCCGAAAGGCCGAA AAAAACAC
 1446 CAGAAAA CUGAUGAGGCCGAAAGGCCGAA AUUUAAA
 1448 AUCAAGU CUGAUGAGGCCGAAAGGCCGAA AUUUAAA
 1449 AAUCAGA CUGAUGAGGCCGAAAGGCCGAA AUUAAA
 1451 UUAUCA CUGAUGAGGCCGAAAGGCCGAA AUAAAGAU
 1456 ACAACUU CUGAUGAGGCCGAAAGGCCGAA AUCAAGAU
 1457 GACAACU CUGAUGAGGCCGAAAGGCCGAA AAUCAGA
 1461 UUAGAC CUGAUGAGGCCGAAAGGCCGAA ACUAAA
 1464 UUGUUUA CUGAUGAGGCCGAAAGGCCGAA ACAACUU
 1466 CAUUGUU CUGAUGAGGCCGAAAGGCCGAA AGACAAC

1479 GUCACCA CUGAUGAGGCCGAAAGGCCGAA AUCAAGCA
1480 GGUCACC CUGAUGAGGCCGAAAGGCCGAA AAUCAGC
1494 AAUGAGU CUGAUGAGGCCGAAAGGCCGAA ACAGUUG
1498 CAGCAAU CUGAUGAGGCCGAAAGGCCGAA AGUGACA
1501 CCUCAGC CUGAUGAGGCCGAAAGGCCGAA AUGAGUG
1512 GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
1517 CCTUGGG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
1528 CAGACAC CUGAUGAGGCCGAAAGGCCGAA ACUCCCC
1533 GAUUAAC A CUGAUGAGGCCGAAAGGCCGAA ACACAAC
1537 GGC CGAU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
1540 GUAGGCC CUGAUGAGGCCGAAAGGCCGAA AUUACAG
1546 UGA AUUAG CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
1549 CAUCGAA CUGAUGAGGCCGAAAGGCCGAA AGUAGGC
1551 GCCACTG CUGAUGAGGCCGAAAGGCCGAA AUAGUAG
1552 CGCCACU CUGAUGAGGCCGAAAGGCCGAA AAUAGUA
1566 CAACCUU CUGAUGAGGCCGAAAGGCCGAA AUUUCUC
1572 CCUAAGC CUGAUGAGGCCGAAAGGCCGAA ACCUUUA
1576 CUUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAACC
1577 UCUUUCC CUGAUGAGGCCGAAAGGCCGAA AAGCAAC

Table 25: Mouse TNF-a HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAUA a GcuCCcA	324	GgGUGAU C GGUCCCC
101	GGCAGGU U CGUGGCC	347	GAGAGGU u CCCAaaU
101	GGCAGGU u CGUGGCC	364	CCUCCTU C UCACUAG
102	GCGGGUU C UgUGCCU	366	UCCTCUU C AUCAGuU
102	gCHAGGUU C ugUGCCU	366	UcccCUU C auCAGuU
106	GUUCGUU C CCUuUCA	369	CUCUcAU C AGUuCUa
110	UgUGCCU u UCAuUCA	376	CAGUuCU A UGGGCCA
111	gUCCCUU u CacUAC	390	AgACCTU C AcacUca
111	guCCCUU u CAcUAC	396	ucaCacU C AGADCAU
112	UcccUUU C AQucACU	401	cUCAGAU C ADCUUCU
116	UuUCACU C AcUGGCC	404	AGADCAU C UUCUCAA
137	GCcaCACU C uCCcUCC	406	AUCADCU U CUCAAAa
139	caCACU C CCUCcAg	406	AUcAUeU U cUcaAAA
177	CCAUGAU C CGcGAG	407	UCAUCUU C UCaAAau
207	AGGGacU C CCCcAAaA	409	AUCUUU U CAAauuC
228	GGGGGUU C CAGAACU	409	AuCuucU C AAAAUUC
228	GGGGGUU c CAGaacU	409	aUcUUeU c AAAauUc
236	CAGaaCU C CAGGGG	432	AGCCUGU A GCCCACG
236	CAGAACU c cAGgcGg		
249	GGUgGCCU a UgUcUcA		
249	GGUgGCCU a UGucUcA	444	AcGUccGU A GCAAACC
		501	AcGCCCCU C CGGGCA
261	UCAAGCCU C UUCUCAU	560	gGgUUGU & CCUugUC
261	UCAgGCCU C UUCUCAu	560	GGguUGU A CCUugUC
263	AGCCUCU U CUCauUC	564	UGUACCU u gUCUACU
263	AgCCUCU U CUCauUC	567	ACCUugU C UACTUCC
264	GCCUCUU C UCaUUCC	569	CUugUCU A CUCCCCAG
264	gCCUCUU C UcauUCC	572	gUCUACU C CCAGGUU
266	CCUCUCU C aUUCUG	572	GUUACU c CCAGguu
269	UUCUCAU U CCUGGUu	572	GUUACU C CCAGGUu
270	UUCUCAUU C CGUGGUg	579	CCCAGGU u CUCUUCA
276	UCCUGU u GUggCAG	580	CCAGguU c UCUUeAa
297	CCACGUU C UUCUGUC	580	CCAGGUU c UCUUeAa
299	ACGUUCU U CGUUCUA	582	AGGUUCU C UUCAagg
300	CGGUUCUU C UGUUCUAc	582	AGGUUCU C UUCAAGG
304	CGUUCGU c UAcUGaa	584	GUuUCUU U CAAGGGa
306	UcUGUcU a cUgAAcU	585	UuUCUUU C AAAGGGaC
314	CGaACU U cGGgGUG	608	CcCGaCU a CgugCUC
315	UGaACUU c GGgGUGA	615	aCgUGcU C CUCACCC
315	uGaaCUU c GGGguGa	615	AcGUGCU C CUCACCC
324	gGGUGaU c GGUCCcC	618	UGCUCUU C ACCACCA

630	ACACCGU C AGCCGau	940	GUCUACU c cUCAGaG
630	ACACCGU C AgCCgaU	943	UACUccU C AGaGcCc
638	agcCgAU u uGCuaUc	972	UCUaaCU u AgAAAGg
643	aUUUGcU a uCUcAaA	972	ucUaaCU u AGAaAgG
645	UuGCuaU C UCaUACC	973	CUaACU A GAAAaggG
647	GUaUCU C aUACCAG	984	AGggGgAU U auGGcuc
663	agAAaGU C AACCUCC	984	AGGGgaU U aUGgcUc
669	UCAACCU C CUUCUUG	985	GGGauU a uGGcUca
669	UcAAccU c cUcUCOG	997	UcAGAaGU c CAACucu
672	ACCUCCU C UCGGCCg	1010	CuguGCU c AGAaCUU
674	CCUCUCU C UGCCgUC	1017	cAGAGCU U UcAACAA
681	cUGCCgU C AagaGcc	1018	AGAGCUU U cAACAC
681	CGGCCgU C AAGAGCC	1019	GAaCUUU c AaCACAC
681	CGGCCgU C aaGAGeC	1073	UggGCCU c ucaUGCA
734	CCCUGGU A UGAGCCC	1096	AAGgAcU C AAAauggG
734	CeeUGGU a ugaGCCc	1106	aUGGGcU U uccGAau
744	AGCCCAU a UACCUUG	1107	UGGGcUU u ccGAauu
746	CCCAAU A CCUGGG	1108	GGgCuUU c cGaaUUC
759	GAaGAGU C uCCAGc	1115	CcGAauU C ACUGGAG
759	GAGGAGU C UUCCAGC	1133	CGAAuugU C CAuucU
761	GGAGUCU U CCAGCUG	1164	gagUGgU c AgGGJUGc
762	GaGUCUU C CAGCUGG	1180	UcUgUeU c agaAUGA
786	ACCAACU C AGCGCUG	1203	aaGAuCU c AGGCCUU
798	CGAGGGU C AAUQuGC	1210	cAGGCCU U CCUaccU
802	GgUCAAU C uGCCCaA	1211	AGGCCUU C CUaccUu
812	CCCAAGU A cuUaGAC	1214	CCUUCU a ccUUCAG
816	AgUAcuU a GACUUUG	1218	CcuACeU u CaGACCU
821	uUaGACU U UGCgGAG	1218	CCuACCU U CAGACCU
822	UaGACUU U GCgGAGU	1218	CCuACCU u cAgACCU
830	GCgGAGU C CGGGCAG	1218	CCUaccU u CAGAccU
840	GGCAGGU C UACUUUG	1219	CuACCUU C AGACCUu
842	CAGGUCU A CUUUGGA	1219	CuACCUU c agACeUU
842	CAGGucU a CUUugGA	1226	CaGACCU U UCCAGAC
842	cagQuCU a CUUugGA	1226	CAGAccU U UCCAGAC
845	GUCUACU U UGGagUC	1227	agACCUU u CCAGACU
846	UCUACUU U GGagUCA	1227	AGAccUU U CCAGACU
852	UUGGagU C AUUGCUC	1228	GAccUUU C CAGACUc
855	GagUCAU U GUUCGU	1238	gACCUU c CCUGAGG
887	AUCCaUU c uctAACCC	1262	CAGCCU U CuCACAG
891	AuucuCU a CCCaGCC	1283	CCCCeeU C uauuuau
905	CCeCaCU C UgaACCC	1283	CCCCCUU C UAUuuAU
905	ccccacU c UgACCCC	1285	ccccCUU A UUUUAuU
905	CCeCACU C UgAcccC	1287	CeUCAU U UauAuUU
914	GAccCCU U uacUCUG	1287	CCUCUAU U UAUauUU
915	ACCCCUU u acUCUGA	1288	CUCIAAU U AUaUJUG
919	CUUUAcU c ugaCCcC	1289	UCUAAUJU A UaUJUGC
928	GACCCCU U UaUugUC	1293	UUUAUJaU U UGCACUU
928	gACCCCU U UAUUguC	1293	UUUJaUaU U UGCACUu
932	CCUUUAU U guQuACU	1294	UUAUaUU U GCACUUa

1300	UUGCACU U aUUAUU	1462	aCCUUGU u GGCUCU
1303	CACUUAU u AUUUAU	1470	GCCUCCU C UUUUGeU
1304	acUUAUU A UUUAUU	1472	cuCcUCU U UUGcUUA
1306	UUAUUAU U UAUUAU	1473	uCcUCUU U UGeUUAU
1307	UAUUAUU U AUUAUU	1474	CeUCUUU U GcUUAUG
1307	UaUUAUU U AuUADuU	1478	UUUUGcU U AUGGUUA
1308	AUUAUOU A UUAAUU	1479	UUUGcUU a UGGuuAA
1310	UauUUAU U AUUUAU	1479	UUUGcUU A UGUUUAa
1310	UAUUAU U AUUUAU	1484	UUAGGUU U aaaAcAA
1310	UAUUAU U AUUUAU	1498	AAAauU U AUCAuAc
1311	AUUAUOU A UUAAUU	1511	AcccAaU U GUCUuAA
1311	AUUAUOU A UUAAUU	1514	caAUUGU C UuAAuAA
1311	AuUUAUU A UUAAUU	1516	aUUGUCU u AAuAACG
1313	UUAUUAU U UAUUAU	1529	CgcugAU u UGGUGAC
1313	UUAUUAU U UAUUAU	1529	CGCTGAGU U UGGUGAC
1313	UUAUUAU U UAUUAU	1530	gCUGAUU u gGUgacc
1314	UAUUAU U AUUUAU	1530	GCUGAUU U GGUGACC
1314	UAUUAU U AUUUAU	1563	UgaAccU c UGeUCCC
1315	AUUAUOU A UUAAUU	1563	ugaacCU C UGCUCCC
1317	UAUUAU U UAUUAU	1568	CUCUGCU C CCCAcGG
1318	AUUAUOU U AUUUAU	1589	UGaCUGU A AUuGeCC
1319	UUUAUOU A UUAAUU	1592	CGUAAAU u GccCUAC
1326	AUUAUOU A UUAAUU	1617	GAGAAA U AAGaUcG
1328	UAUUAU U UAUUgC	1623	UAAAGaU c GCUUAAA
1329	AUUAUOU U AUUUGCu	1633	UUaaaaU a aaAAaacc
1330	UUUAUOU A UUUGCu	25	AgGgaCU a gCCagGA
1332	UAUUAU U UgCuAU		
1333	AUUAUOU U gCuuADG		
1337	auUUGCU U AUGAAuG		
1338	UJUGCUU A ugAAuGu		
1346	UGAAUGU A UUAAUU		
1348	AADGUAU U UAUUOGG		
1349	ADGUAUU U AUUUGGA		
1350	UGUAAUU A UUUGGA		
1352	UADGUAU U UGGaAGG		
1352	UADGUAU U UGGaAGG		
1353	AUUAUOU U GGaAGgC		
1369	GGGGUgU C CUGGAGG		
1398	gCUGUCU U cAGACAG		
1398	GGUGUCU U cagaCAG		
1412	GACAGGU U UUCUgUG		
1413	ACADGUU U UCUUGGA		
1414	CAUGGUU U CuGUGAA		
1415	ADGUUUU C uGUGAAA		
1415	ADGUUUU C UguGAA		
1438	gaGCGGU C CCCAccU		
1451	CGGGCU C UeUaCCU		
1453	ggCCUCU C UaCCuUG		

Table 26: Mouse TNF- α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUCGGC CUGAUGAGGCGGAAGGCGAA AGUCCU
66	UGGGAGC CUGAUGAGGCGGAAGGCGAA AUUUCCA
101	GGGACAG CUGAUGAGGCGGAAGGCGAA ACCUGCC
101	GGGACAG CUGAUGAGGCGGAAGGCGAA ACCUGCC
102	AGGGACA CUGAUGAGGCGGAAGGCGAA AACCTUGC
102	AGGGACA CUGAUGAGGCGGAAGGCGAA AACCTUGC
106	UGAAAGG CUGAUGAGGCGGAAGGCGAA ACAGAAC
110	UGAGUGA CUGAUGAGGCGGAAGGCGAA AGGGACA
111	GUGAGUG CUGAUGAGGCGGAAGGCGAA AAGGGAC
111	GUGAGUG CUGAUGAGGCGGAAGGCGAA AAGGGAC
112	AUGAGAU CUGAUGAGGCGGAAGGCGAA AAAGGGGA
115	GGCCAGU CUGAUGAGGCGGAAGGCGAA AGUGAAA
137	GGAGGGA CUGAUGAGGCGGAAGGCGAA AUGGGGC
139	CUGGAGG CUGAUGAGGCGGAAGGCGAA AGAUUGG
177	CGUUCGG CUGAUGAGGCGGAAGGCGAA AUCAUDG
207	UUUGGGG CUGAUGAGGCGGAAGGCGAA AGUGOCU
228	AGUUCUG CUGAUGAGGCGGAAGGCGAA AAGCCCC
228	AGUUCUG CUGAUGAGGCGGAAGGCGAA AAGCCCC
236	CGCCUCG CUGAUGAGGCGGAAGGCGAA AGTUCUG
236	CGCCUCG CUGAUGAGGCGGAAGGCGAA AGTUCUG
249	UGAGACA CUGAUGAGGCGGAAGGCGAA AGGCACC
249	UGAGACA CUGAUGAGGCGGAAGGCGAA AGGCACC
261	AUGAGAA CUGAUGAGGCGGAAGGCGAA AGGTUGA
261	AUGAGAA CUGAUGAGGCGGAAGGCGAA AGGCUGA
263	GAADGAG CUGAUGAGGCGGAAGGCGAA AGAGGCC
263	GAADGAG CUGAUGAGGCGGAAGGCGAA AGAGGCC
264	GGAAUUGA CUGAUGAGGCGGAAGGCGAA AAGAGGC
264	GGAAUUGA CUGAUGAGGCGGAAGGCGAA AAGAGGC
266	CAGGUAU CUGAUGAGGCGGAAGGCGAA AGRAGAG
269	AAGCAGG CUGAUGAGGCGGAAGGCGAA AUGAGAA
270	CAAGCAG CUGAUGAGGCGGAAGGCGAA AADGAGA
276	CUGCCAC CUGAUGAGGCGGAAGGCGAA AGCAGGA
297	GACAGAA CUGAUGAGGCGGAAGGCGAA AGCGUUG
299	UAGACAG CUGAUGAGGCGGAAGGCGAA AGAGCCU
300	GUAGACAA CUGAUGAGGCGGAAGGCGAA AAGAGCG
304	UUCAGUA CUGAUGAGGCGGAAGGCGAA ACAGAAG
306	AGGUUCAG CUGAUGAGGCGGAAGGCGAA AGACAGA
314	CAACCCC CUGAUGAGGCGGAAGGCGAA AGUUCAG
315	UCACCCC CUGAUGAGGCGGAAGGCGAA AAGUUCAG

315	UCACCCU CUGAUGAGGCGAAAGGCCGAA AAGGUCA
324	GGGGACC CUGAUGAGGCGAAAGGCCGAA AUCAACC
324	GGGGACC CUGAUGAGGCGAAAGGCCGAA AUCAACC
347	AUUUGGG CUGAUGAGGCGAAAGGCCGAA ACUUCUC
364	CTGAGGA CUGAUGAGGCGAAAGGCCGAA AGGGAGG
366	AACUGAU CUGAUGAGGCGAAAGGCCGAA AGAGGGA
366	AACUGAU CUGAUGAGGCGAAAGGCCGAA AGAGGGA
369	UAGAACU CUGAUGAGGCGAAAGGCCGAA AGGAGAG
376	UGGGCGA CUGAUGAGGCGAAAGGCCGAA AGAACCG
390	UGAGGCU CUGAUGAGGCGAAAGGCCGAA AGGGCCU
396	ADGACCU CUGAUGAGGCGAAAGGCCGAA AGGUGUA
401	AGAAGAU CUGAUGAGGCGAAAGGCCGAA AGCCGAG
404	UUGAGAU CUGAUGAGGCGAAAGGCCGAA AGGACCU
406	UUUUGAG CUGAUGAGGCGAAAGGCCGAA AGAUGAU
406	UUUUGAG CUGAUGAGGCGAAAGGCCGAA AGAUGAU
407	ADUUGGA CUGAUGAGGCGAAAGGCCGAA AGAGAGA
409	GAADUUU CUGAUGAGGCGAAAGGCCGAA AGAAGAU
409	GAADUUU CUGAUGAGGCGAAAGGCCGAA AGAAGAU
409	GAADUUU CUGAUGAGGCGAAAGGCCGAA AGAAGAU
432	CGUGGGC CUGAUGAGGCGAAAGGCCGAA AGGGCCU
444	GGUUGGC CUGAUGAGGCGAAAGGCCGAA ACCGGU
501	UGGOCAG CUGAUGAGGCGAAAGGCCGAA AGGGCGU
560	GACRAGG CUGAUGAGGCGAAAGGCCGAA ACRAACC
560	GACRAGG CUGAUGAGGCGAAAGGCCGAA ACRAACC
564	AGTAGAC CUGAUGAGGCGAAAGGCCGAA AGGTACA
567	GGGAGUA CUGAUGAGGCGAAAGGCCGAA ACRAAGU
569	CUGGGAG CUGAUGAGGCGAAAGGCCGAA AGACAG
572	AAACUUG CUGAUGAGGCGAAAGGCCGAA AGTAGAC
572	AAACUUG CUGAUGAGGCGAAAGGCCGAA AGTAGAC
572	AAACUUG CUGAUGAGGCGAAAGGCCGAA AGTAGAC
579	UGAAGAG CUGAUGAGGCGAAAGGCCGAA ACCUGGG
580	UUGAAGA CUGAUGAGGCGAAAGGCCGAA AACCUUG
580	UUGAAGA CUGAUGAGGCGAAAGGCCGAA AACCUUG
582	CCUUGAA CUGAUGAGGCGAAAGGCCGAA AGAACCU
582	CCUUGAA CUGAUGAGGCGAAAGGCCGAA AGAACCU
584	UCCCUUG CUGAUGAGGCGAAAGGCCGAA AGAGAAC
585	GUCCCCU CUGAUGAGGCGAAAGGCCGAA AAGAGAA
608	GAGCACG CUGAUGAGGCGAAAGGCCGAA AGUUGGG
615	GGUUGAG CUGAUGAGGCGAAAGGCCGAA AGCACGU
615	GGUUGAG CUGAUGAGGCGAAAGGCCGAA AGCACGU
618	UGUGGGU CUGAUGAGGCGAAAGGCCGAA AGGRGCA
630	AUCGGCU CUGAUGAGGCGAAAGGCCGAA ACGGUGU
630	AUCGGCU CUGAUGAGGCGAAAGGCCGAA ACGGUGU
638	GAUAGCA CUGAUGAGGCGAAAGGCCGAA ADGGCU
643	UAUGAGA CUGAUGAGGCGAAAGGCCGAA AGCAAAU
645	GGTAUGA CUGAUGAGGCGAAAGGCCGAA ATAGCAA
647	CGGUAU CUGAUGAGGCGAAAGGCCGAA AGAIDGC

663 GGAGGUU CUGAUGAGGCCGAAAGGCCGAA ACUUTCU
 669 CAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGGJUGA
 669 CAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGGJUGA
 672 CGGCAGA CUGAUGAGGCCGAAAGGCCGAA AGGAGGU
 674 GACGGCA CUGAUGAGGCCGAAAGGCCGAA AGAGGGAG
 681 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA ACGGCAG
 681 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA ACGGCAG
 734 GGGCUCA CUGAUGAGGCCGAAAGGCCGAA ACCRAGGG
 734 GGGCUCA CUGAUGAGGCCGAAAGGCCGAA ACCRAGGG
 744 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA ADGGGCJ
 746 UCCCGGG CUGAUGAGGCCGAAAGGCCGAA ACGTGGG
 759 GCUUGGA CUGAUGAGGCCGAAAGGCCGAA ACTCCTC
 759 GCUUGGA CUGAUGAGGCCGAAAGGCCGAA ACTCCTC
 761 CAGCTGG CUGAUGAGGCCGAAAGGCCGAA AGACTCC
 762 CCAGCTG CUGAUGAGGCCGAAAGGCCGAA AAGACTC
 786 CAGCGTJ CUGAUGAGGCCGAAAGGCCGAA AGUUGGU
 798 GCAAGAU CUGAUGAGGCCGAAAGGCCGAA ACCUCAG
 802 UGGGGCA CUGAUGAGGCCGAAAGGCCGAA AUUGACC
 812 GCUUAG CUGAUGAGGCCGAAAGGCCGAA ACTUUGG
 816 CAAAGUC CUGAUGAGGCCGAAAGGCCGAA AAGUACJ
 821 CUCCGCA CUGAUGAGGCCGAAAGGCCGAA AGUCCTA
 822 ACUCCGC CUGAUGAGGCCGAAAGGCCGAA AAGUCCTA
 830 CGGCGCG CUGAUGAGGCCGAAAGGCCGAA ACTUCGC
 840 CAAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUCGC
 842 UCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCTG
 842 UCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCTG
 842 UCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCTG
 845 GACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 846 UGACTCC CUGAUGAGGCCGAAAGGCCGAA AAGIAGA
 852 GAGCAU CUGAUGAGGCCGAAAGGCCGAA ACTUCRA
 855 ACAGAGC CUGAUGAGGCCGAAAGGCCGAA ADGACTC
 887 GGGUAGA CUGAUGAGGCCGAAAGGCCGAA AADGGAU
 891 GGCUGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAJ
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 914 CAGAGUA CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
 915 UCAAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGGGU
 919 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUAAAAG
 928 GACAATA CUGAUGAGGCCGAAAGGCCGAA AGGGGJC
 928 GACAATA CUGAUGAGGCCGAAAGGCCGAA AGGGGJC
 932 AGUAGAC CUGAUGAGGCCGAAAGGCCGAA AUAAGG
 940 CUCUGAG CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 943 GGGCUCU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
 972 CCUUCUCU CUGAUGAGGCCGAAAGGCCGAA AGUUAGA
 972 CCUUCUCU CUGAUGAGGCCGAAAGGCCGAA AGUUAGA
 973 CCCUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUJAG
 984 GAGCCAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCU

984	GAGCCAU CUGAUGAGGCCGAAAGGCGAA AUCCCCU
985	UGAGCCA CUGAUGAGGCCGAAAGGCGAA AAUCCCC
997	AGAGUUG CUGAUGAGGCCGAAAGGCGAA ACUUCGA
1010	AAGCUCU CUGAUGAGGCCGAAAGGCGAA AGCAACG
1017	UUGGUUG CUGAUGAGGCCGAAAGGCGAA AGCUCUG
1018	GUUGUUG CUGAUGAGGCCGAAAGGCGAA AAGCUCU
1019	AGUUGUU CUGAUGAGGCCGAAAGGCGAA AAAGCUC
1073	UCCADGA CUGAUGAGGCCGAAAGGCGAA AGGCCCA
1096	CCCAUUA CUGAUGAGGCCGAAAGGCGAA AGUCCUU
1106	AUUCGGA CUGAUGAGGCCGAAAGGCGAA AGCCCAU
1107	AAUUCGG CUGAUGAGGCCGAAAGGCGAA AAGCCCA
1108	GAATUCG CUGAUGAGGCCGAAAGGCGAA AAAGCCC
1115	CCUCAGU CUGAUGAGGCCGAAAGGCGAA AAUUCGG
1133	AGGAADG CUGAUGAGGCCGAAAGGCGAA ACACUCG
1164	GCAACCU CUGAUGAGGCCGAAAGGCGAA ACCACDC
1180	UCAUCU CUGAUGAGGCCGAAAGGCGAA AGAACGA
1203	AAGGCCU CUGAUGAGGCCGAAAGGCGAA AGAACUU
1210	AGGUAGG CUGAUGAGGCCGAAAGGCGAA AGGCCCCG
1211	AAGGUAG CUGAUGAGGCCGAAAGGCGAA AAGGCCU
1214	CUGAAGG CUGAUGAGGCCGAAAGGCGAA AGGAAGG
1218	AGGUUCG CUGAUGAGGCCGAAAGGCGAA AGGUAGG
1219	AGGUUCU CUGAUGAGGCCGAAAGGCGAA AGGUAGG
1219	AAGGUUCU CUGAUGAGGCCGAAAGGCGAA AAGGUAG
1226	GUCCUGGA CUGAUGAGGCCGAAAGGCGAA AGGUUCG
1226	GUCCUGGA CUGAUGAGGCCGAAAGGCGAA AGGUUCG
1227	AGUCUGG CUGAUGAGGCCGAAAGGCGAA AAGGUUC
1227	AGUCUGG CUGAUGAGGCCGAAAGGCGAA AAGGUUC
1228	GAGUCUG CUGAUGAGGCCGAAAGGCGAA AAGGUUC
1238	CCUCAGG CUGAUGAGGCCGAAAGGCGAA AAGGUUC
1262	CUGUGAG CUGAUGAGGCCGAAAGGCGAA AAGGUUC
1283	AATAATA CUGAUGAGGCCGAAAGGCGAA AGGGGGG
1283	AATAATA CUGAUGAGGCCGAAAGGCGAA AGGGGGG
1285	AATAATA CUGAUGAGGCCGAAAGGCGAA AGGGGGG
1287	AAAATAA CUGAUGAGGCCGAAAGGCGAA ATAGAGG
1287	AAAATAA CUGAUGAGGCCGAAAGGCGAA ATAGAGG
1288	CAAAUAU CUGAUGAGGCCGAAAGGCGAA AATAGAG
1289	GCAAAUUA CUGAUGAGGCCGAAAGGCGAA AATAGAG
1293	AAGUGCA CUGAUGAGGCCGAAAGGCGAA AATAAAA
1293	AAGUGCA CUGAUGAGGCCGAAAGGCGAA AATAAAA
1294	UAGUGGC CUGAUGAGGCCGAAAGGCGAA AATAAAA
1300	AAAATAAU CUGAUGAGGCCGAAAGGCGAA AGUGCAA
1303	AATAAAAU CUGAUGAGGCCGAAAGGCGAA AUAAGCG
1304	UAAAATA CUGAUGAGGCCGAAAGGCGAA AATAAGG
1306	AATAAAAU CUGAUGAGGCCGAAAGGCGAA AUAATAA
1307	AAAATAAU CUGAUGAGGCCGAAAGGCGAA AUAATAA
1307	AAAATAAU CUGAUGAGGCCGAAAGGCGAA AUAATAA

1308 CGAAGGCGAAAGGCCGAA AAATAAU
 1310 CGAAGGCGAAAGGCCGAA ATAAAU
 1310 CGAAGGCGAAAGGCCGAA ATAAATA
 1310 CGAAGGCGAAAGGCCGAA ATAAATA
 1311 CGAAGGCGAAAGGCCGAA ATAAAU
 1311 CGAAGGCGAAAGGCCGAA ATAAAU
 1311 CGAAGGCGAAAGGCCGAA ATAAAU
 1313 CGAAGGCGAAAGGCCGAA ATAAATA
 1313 CGAAGGCGAAAGGCCGAA ATAAATA
 1313 CGAAGGCGAAAGGCCGAA ATAAATA
 1314 CGAAGGCGAAAGGCCGAA ATAAATA
 1314 CGAAGGCGAAAGGCCGAA ATAAATA
 1315 CGAAGGCGAAAGGCCGAA ATAAAU
 1317 CGAAGGCGAAAGGCCGAA ATAAATA
 1318 CGAAGGCGAAAGGCCGAA ATAAAU
 1319 CGAAGGCGAAAGGCCGAA ATAAAU
 1326 CGAAGGCGAAAGGCCGAA ATAAAU
 1328 CGAAGGCGAAAGGCCGAA ATAAATA
 1329 CGAAGGCGAAAGGCCGAA ATAAAU
 1330 CGAAGGCGAAAGGCCGAA ATAAAU
 1332 CGAAGGCGAAAGGCCGAA ATAAATA
 1333 CGAAGGCGAAAGGCCGAA ATAAAU
 1337 CGAAGGCGAAAGGCCGAA AGCAAU
 1338 CGAAGGCGAAAGGCCGAA AGCAAA
 1346 CGAAGGCGAAAGGCCGAA ACATCA
 1348 CGAAGGCGAAAGGCCGAA ATACAU
 1349 CGAAGGCGAAAGGCCGAA ATACAU
 1350 CGAAGGCGAAAGGCCGAA ATACAU
 1352 CGAAGGCGAAAGGCCGAA ATAAAU
 1352 CGAAGGCGAAAGGCCGAA ATAAAU
 1353 CGAAGGCGAAAGGCCGAA ATAAAU
 1369 CGAAGGCGAAAGGCCGAA ATAAAU
 1398 CGAAGGCGAAAGGCCGAA AGACAGC
 1398 CGAAGGCGAAAGGCCGAA AGACAGC
 1412 CGAAGGCGAAAGGCCGAA ACAGUC
 1413 CGAAGGCGAAAGGCCGAA AACAGU
 1414 CGAAGGCGAAAGGCCGAA AACAGD
 1415 CGAAGGCGAAAGGCCGAA AAAACAU
 1415 CGAAGGCGAAAGGCCGAA AAAACAU
 1438 CGAAGGCGAAAGGCCGAA ACAGCUC
 1451 CGAAGGCGAAAGGCCGAA AGGCCAG
 1453 CGAAGGCGAAAGGCCGAA AGAGGCC
 1455 CGAAGGCGAAAGGCCGAA AGAGAGG
 1462 CGAAGGCGAAAGGCCGAA ACAAGGU
 1470 CGAAGGCGAAAGGCCGAA AGGAGGC
 1472 CGAAGGCGAAAGGCCGAA AGAGGAG
 1473 CGAAGGCGAAAGGCCGAA AAGAGGA
 1474 CGAAGGCGAAAGGCCGAA AAGAGGG
 1478 CGAAGGCGAAAGGCCGAA AGCAAAA

1479	UUAAAACA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
1479	UUAAAACA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
1484	UUGUUUU CUGAUGAGGCCGAAAGGCCGAA AACAUAA
1498	GUAAGAU CUGAUGAGGCCGAAAGGCCGAA AAIAAUU
1511	UUAAGAC CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
1514	UUAUAAA CUGAUGAGGCCGAAAGGCCGAA ACAAUUG
1516	CGUUAUU CUGAUGAGGCCGAAAGGCCGAA AGACAAU
1529	GUCACCA CUGAUGAGGCCGAAAGGCCGAA AUCAAGG
1529	GUCACCA CUGAUGAGGCCGAAAGGCCGAA AUCAAGG
1530	GGUCACC CUGAUGAGGCCGAAAGGCCGAA AADCAGC
1530	GGUCACC CUGAUGAGGCCGAAAGGCCGAA AADCAGC
1563	GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGGUUCA
1563	GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGGUUCA
1568	CGUGGGG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
1589	GGGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAGUCA
1592	GTAGGGC CUGAUGAGGCCGAAAGGCCGAA AUUACAG
1617	CGAUUU CUGAUGAGGCCGAAAGGCCGAA AUUOCUC
1623	UUAAGC CUGAUGAGGCCGAAAGGCCGAA AUCCUUA
1633	GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA

Table 27: Human TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	ACCCGUGG AGAA GUAGUCU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	ACAUACU GAC CCAAGGGCU
54	GAGGGUGG AGAA GUUGGUU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	ACCCACG GCU CCAACCCUC
105	GGAGAGAGA AGAA GGGGA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UUCCUCA GCC UCUUCUCC
201	CUGGCCAGC AGAA GGAGG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CCUUCU GAU CGUUGGAG
230	GUGGAGGA AGAA GAAGG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CUCUUCU GCC UGGUGGAC
234	CAAAGUGC AGAA GCGAGA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UCUGCCU GCU GCACUUNG
254	CCUCUGGG AGAA GUUCAC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GUGAUCG GCC CCCAGAGG
296	GGCCAGAG AGAA GUUDG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CURAUCA GCC CUCUGGCC
317	AGAAGAGU AGAA GACUCG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GGAGUCA GAU CAUCUUCU
387	GCCACUGG AGAA GCCCCU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	AGGGGCA GCU CCAGUGGC
404	AUUGGCC AGAA GUUCAG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UGRAACC GCC GCGCCAAU
453	GGACCCAC AGAA GGUUAU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	AUNACCA GCU AGUGGGGC
518	GGUGGAGG AGAA GCCUUC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CAAGGU GGC CCUCACCC
554	GGCGAUGC AGAA GUUCGU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	ACCAUCA GGC GCUUCGCC
565	UGGUAGGA AGAA GGCAUG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CAUCGC GUC UCUUACCA
576	UGACCUUAG AGAA GGUAGG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CCUACCA GAC CAGGGUCA
607	CCUCUCUC AGAA GGAGGA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UCUUCCA GCU GGAGAGG
704	AGCGCUCA AGAA GUUCAC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GGUGAAC GAC UCAGGCCU
726	GAUAGUCG AGAA GUUUGA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UCAAUCA GGC CGACAUAC
730	UCGAGAUAG AGAA GGCGGA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	UCTGGCC GAC UUUCUGA
824	GGGAGUUGG AGAA GGCGAG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CUUCUCU GGC CCAAUCC
1042	GGGAUUCUA AGAA GUAGGC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GCCUACA GCU UUGAUCC
1168	CUGGAAC AGAA GGAGAG ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	CUCUCCA GAU GUUCCAG
1178	UCAGGAA AGAA GGAAAC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GUUCCA GAC UUCCUGA
1202	AUGGGGAG AGAA GGGCUC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GAGGCCA GGC CUCCCCAU
1220	AUAGAGGG AGAA GGCUCC ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	GGAGCCA GCU CCUCUCAU
1284	AUACAUUC AGAA GUAAA ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	AUUACA GAU GAAGUUAU
1340	UGAGCCAA AGAA GCUCCU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	AGGAGCU GCC UGGGUCAU
1390	UACAUUGG AGAA GCUUAU ACCAGAGAAACACACGUUUCGUUACAUUACCUUGUA	AUAGGUU GUI CCCAUGUA

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1452 ACATCAUA AGAA GUAAA ACCAGAGAACACAGGUUACAUUACCGGUA AUUUCU GAA UACGUU
 1475 GUCACCAAA AGAA GCUUUG ACCAGAGAACACAGGUUACAUUACCGGUA CAAUCU GAA UGGGAC
 1513 CCUCUGGG AGAA GGGCC ACCAGAGAACACAGGUUACAUUACCGGUA GCCCUU GCU OCCTGGG
 1541 GAAUAGUA AGAA GAUAC ACCAGAGAACACAGGUUACAUUACCGGUA GUAAUC GGC UCCUAC

Table 28: Mouse TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GCGGAGC AGAA GCGCU ACCAGGAAACACAGGGUACAUUACUGGUA	ACGUUCU GUC ACUUCAC
256	UGAGAAGA AGAA GAGCA ACCAGGAAACACAGGGUACAUUACUGGUA	UGUCUCA GGC UCUUCUA
272	CUCCACCA AGAA GGAAAG ACCAGGAAACACAGGGUACAUUACUGGUA	CAUUCU GGU UGGGGAG
301	GUCCCCCC AGAA GAGGG ACCAGGAAACACAGGGUACAUUACUGGUA	CUCUUCU GUC UACGGAC
325	CCUUUUGG AGAA GAUCAC ACCAGGAAACACAGGGUACAUUACUGGUA	GUGAUGG GUC OCACAGG
370	CCCCATTA AGAA GAGGG ACCAGGAAACACAGGGUACAUUACUGGUA	CUCAUCU GUU CTAUCCC
383	GUGUGAGG AGAA GCGCA ACCAGGAAACACAGGGUACAUUACUGGUA	UCCGCGA GAC COUCAC
397	AGAGAGAG AGAA GAGCU ACCAGGAAACACAGGGUACAUUACUGGUA	ACAGCUA GAU GAGCUUCU
467	GGCACUCC AGAA GCGCU ACCAGGAAACACAGGGUACAUUACUGGUA	ACGAGCA GCU GCGGCC
546	APOCCACU AGAA GCGCC ACCAGGAAACACAGGGUACAUUACUGGUA	GGCCGCA GCC GAGGGG
549	UACACCC AGAA GCGGC ACCAGGAAACACAGGGUACAUUACUGGUA	GCGGCCC GAU GCGGCGA
598	GUGUGGG AGAA GCGUG ACCAGGAAACACAGGGUACAUUACUGGUA	CGAGGCU GGC GCGACUC
603	AGCACGUA AGAA GCGCG ACCAGGAAACACAGGGUACAUUACUGGUA	CGCCCGC GAC UAGGGCU
631	ACCAAAUC AGAA GCGGGU ACCAGGAAACACAGGGUACAUUACUGGUA	ACGGGUA GGC GAGUUCU
634	GUAGCCAA AGAA GCGUCG ACCAGGAAACACAGGGUACAUUACUGGUA	GUGACCC GAU UGGCGAC
675	CUCUUCAC AGAA GAGGG ACCGGGGACACGGGACAUUACUGGUA	CCUCUUCU GGC GCAAGAG
691	GUCCUGGG AGAA GCGGGU ACCAGGAAACACAGGGUACAUUACUGGUA	AGGGCUU GGC OCACGAC
764	CCUCUCC AGAA GGAGA ACCGGGAAACACGGGACAUUACUGGUA	UCUUCU A GCU CGGAGAC
803	AGUACUG AGAA GGUUG ACCGGGAAACACGGGACAUUACUGGUA	UCAUCU GGC CGAUUCU
895	AGACGUCC AGAA GCGGG ACCGGGAAACACGGGACAUUACUGGUA	CGUACCUA GGC CGAUUUAU
906	GUUAGGG AGAA GCGGG ACCGGGAAACACGGGACAUUACUGGUA	GGCGGCUA GUC UGCUUCU
920	AUAAAAGG AGAA GCGGA ACCGGGAAACACGGGACAUUACUGGUA	UCCUCU GGC UCGAGAG
953	AGGAGACA AGAA GCGGC ACCGGGAAACACGGGACAUUACUGGUA	ACCUCA GAC CGUUCAG
1175	CUUCUGA AGAA GCGCA ACCGGGAAACACGGGACAUUACUGGUA	CUUUCU GAC UGCUUCU
1220	CUGGAGAG AGAA GAGGU ACCGGGAAACACGGGACAUUACUGGUA	ACCUCA GAC CGUUCAG
1230	ACGGGAGA AGAA GCGAU ACCGGGAAACACGGGACAUUACUGGUA	CUUUCU GAC UGCUUCU
1256	GUCCAGCA AGAA GCGAU ACCGGGAAACACGGGACAUUACUGGUA	ACUUCHA GGC UGCUUCU
1274	UAGGGGG AGAA GCGCU ACCGGGAAACACGGGACAUUACUGGUA	AGGGGGCA GGC UGCUUCU

1393	GGCGGAA AGCA GCGUUU ACCGGGUAGGCUUACUUCUUA GGCGGAA AGCA GCGUUU ACCGGGUAGGCUUACUUCUUA
1435	CGGGGG AGCA GCGGUU ACCGGGUAGGCUUACUUCUUA
1525	GUCCCCA AGCA GCGGUU ACCGGGUAGGCUUACUUCUUA
1542	GUCCCCC AGCA GCGGUU ACCGGGUAGGCUUACUUCUUA
1564	CGGGGG AGCA GCGGUU ACCGGGUAGGCUUACUUCUUA

Table 29: Human *bcr/abl* HH Target Sequence

Sequence ID N .	HH Target Sequence
<u><i>b2-a2</i></u> <u>Junction</u>	
20	UGACCAUCA AUU AGGAGAGGCC
21	GAAGAAGCC CUU CAGGGGCGAGU
22	AAGRAAGCCC UUC AGGGGGCGAGUA
<u><i>b3-a2</i></u> <u>Junction</u>	
23	UAGGCGAG UUC AAAAGCCCCUUC
24	UCAAAAGCC CUU CAGGGGCGAGU
25	CAAAAGCCC UUC AGGGGGCGAGUA

Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GGCUUCUUCU CGAUGAGCCCGAAAGGCGAA AUUGAUCCUA
27	ACUGGCCGCTG CGAUGAGCCCGAAAGGCGAA AGGGCUCUUC
28	UACTGGCCGT CGAUGAGCCCGAAAGGCGAA AAGGGCUUCUU
29	GAAGGGCUUU CGAUGAGCCCGAAAGGCGAA AACUCUGCUUA
30	ACUGGCCGCTG CGAUGAGCCCGAAAGGCGAA AGGGCUCUUUGA
31	UACTGGCCGT CGAUGAGCCCGAAAGGCGAA AAGGGCUUUUG

Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAU A AAUCAAU	276	AAAAAUU A CUGAAUR
14	AADAAAU C AAUDCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CAGCCUA	295	ACAAAAAU A UGGCACU
19	AUCAAAU C AGCCAC	303	UGGCACU U UCCCCAU
54	CAAGAU A AUACACC	304	GCCACUU U CCCUAG
57	UGAATAAU A CACCACA	305	GCACUUU C CCTTAUGC
77	UGAGGAAU C ACAGACA	309	UUUCCCU A UGCCAU
94	AGACCGU U GUCACUU	317	UGCCAUU A UUCATCA
97	CCGUUGU C ACUTUGAG	319	CCAAAUU U CRUCAAU
101	UGUCACU U GAGACCA	320	CAATAAU C AUCAAC
110	AGACCAU A ATAACAU	323	TAUCAAU C AADCAUG
113	CCATAAU A ACACAC	327	CAUCAAU C AUGAUUG
118	ATAACAU C ACUAACC	337	GAUGGGU U CUTAGAA
122	CAUCACU A ACCAGAG	338	AUUGGUU C UUAGAAU
134	GAGACAU C ATAACAC	340	GGGUUCU U AGAAUGC
137	ACACCAU A ACACACA	341	GGUCCUU A GAAUGCA
148	CACAAAU U UAAUAC	350	AAGCAU U GGCAUUA
149	ACAAAUU U AUAUACU	356	UCCGGCAU U AAGCCUA
150	CAAAUOU A UAAUACU	357	GGGCAUU A AGCCUAC
152	AAUUAU A UACUOGA	363	UAAGCCU A CAAAGCA
154	UUUAAU A CUUGADA	372	AAAGCAU A CUCCAU
157	AUAAUACU U GAUAAA	375	GCACAU C CCATAAU
161	ACUUGAU A AADCAG	380	CUCACAU A AUAAUACA
165	GAUAAAU C ADGAAG	383	CCAUAAU A UACAGU
176	AAUGCAU A GUGAGAA	385	AUAAUAU A CAAGUAU
188	GAACACU U GAUGAAA	391	UACAAGU A UGAUUC
208	GGCACAU U UACAUUC	396	GUAGAU C UCAAUCC
209	CCACAUU U ACACUCC	398	AUGAUCU C AADCCAU
210	CACAUU A CAUCCU	402	UCUCAU C CAUAAAU
214	UUUACAU U CCUGGUC	406	AAUCCAU A AAUUCA
215	UUACAUU C CUGGUCA	410	CAUAAAU U UCAACAC
221	UCCUGGU C AACUADG	411	AUAAAUU U CAACACA
226	GUCAACU A UGAAADG	412	UAAAADU C AACACAA
239	UGAAACU A UUACACA	421	ACACAAU A UUCACAC
241	AAACTAU U ACACAAA	423	ACAAUAU U CACACAA
242	AACTAUU A CACAAAG	424	CAATAAU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCAAU A AAUAAAU	434	ACAAUCU A AAACAAC
265	ACUAAAUAU A UAAAAAA	446	AACAAUCU C UAUCCAU
267	UAAAUAU A AAAAADA	448	CAACUCU A UGCAUAA
274	AAAAAAU A UACUGAA	454	UAGGCAU A ACUAC

458	CATAACU A UACUCCA
460	UAACAUU A CUCCAUA
463	CUAUCU C CAUCGUC
467	AUCUCAU A GUCCAGA
470	CCAUAGU C CAGADGG
489	UGAAAAU U ADAGUAA
490	GAAAADU A UAGUAAU
492	AAAUAU A GUAADUU
495	UUAUAGU A AUUAAA

Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGAUU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
14	CUGAAUU CUGAUGAGGCCGAAAGGCCGAA AUUUAUU
18	UUGGCTG CUGAUGAGGCCGAAAGGCCGAA AUUGAUU
19	GUUGGCU CUGAUGAGGCCGAAAGGCCGAA AUUGAU
54	GGUGUAU CUGAUGAGGCCGAAAGGCCGAA AUCAUUG
57	UGUGGGUG CUGAUGAGGCCGAAAGGCCGAA AUUAUCA
77	UGUCUGU CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
94	AAGUGAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCU
97	CUCAAGU CUGAUGAGGCCGAAAGGCCGAA ACAACGG
101	UGGUUCU CUGAUGAGGCCGAAAGGCCGAA AGUGACA
110	AUGUUAU CUGAUGAGGCCGAAAGGCCGAA AUGGUU
113	GUGADGU CUGAUGAGGCCGAAAGGCCGAA AUUADGG
118	GGUUAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUAU
122	CUCUGGU CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
134	GUGUUAU CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
137	UGUGUGU CUGAUGAGGCCGAAAGGCCGAA AUGUUGU
148	GUADADA CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
149	AGUUAUA CUGAUGAGGCCGAAAGGCCGAA AAUTUGU
150	AAGUUAU CUGAUGAGGCCGAAAGGCCGAA AAUUTUG
152	UCAAGUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
154	UATCAAG CUGAUGAGGCCGAAAGGCCGAA ACUTAAA
157	AUUTAUC CUGAUGAGGCCGAAAGGCCGAA AGUTAUA
161	CAUGAUU CUGAUGAGGCCGAAAGGCCGAA AUCAAGU
165	CAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUUTAC
176	UUCUCAC CUGAUGAGGCCGAAAGGCCGAA AUGCAU
188	UUUCUAC CUGAUGAGGCCGAAAGGCCGAA AGUUUUC
208	GAADGTA CUGAUGAGGCCGAAAGGCCGAA AUGUGGC
209	GGAADGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
210	AGGAADG CUGAUGAGGCCGAAAGGCCGAA AAAUGUG
214	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AUGUAAA
215	UGACCAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAA
221	CAAGAUU CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
226	CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUUGAC
239	UGUGUAA CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
241	UUUGUGU CUGAUGAGGCCGAAAGGCCGAA AUAGUUU
242	CUUUGUG CUGAUGAGGCCGAAAGGCCGAA AAUAGUU
251	UGGUUCC CUGAUGAGGCCGAAAGGCCGAA ACUUUUGU
261	UUAUAAU CUGAUGAGGCCGAAAGGCCGAA AGUGGUU
265	UUUUUUA CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
267	UAUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAUUUA
274	UUCAGUA CUGAUGAGGCCGAAAGGCCGAA AUUUUUU
276	UAUUCAG CUGAUGAGGCCGAAAGGCCGAA AUAUUUA

283 UGGUGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCAGU
 295 AGUGCCA CUGAUGAGGCCGAAAGGCCGAA AUUUTUGU
 303 AUAGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGCCA
 304 CAUAGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCC
 305 GCAGTAGG CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
 309 AUUGGCA CUGAUGAGGCCGAAAGGCCGAA AGGGAAA
 317 UGAUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
 319 AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUUUTGG
 320 GAUUGAU CUGAUGAGGCCGAAAGGCCGAA AAUATUG
 323 CAUGAUU CUGAUGAGGCCGAAAGGCCGAA ADGAAUA
 327 CCUCUAC CUGAUGAGGCCGAAAGGCCGAA ADGAAOG
 337 UUCUAG CUGAUGAGGCCGAAAGGCCGAA ACCAACC
 338 AUUCUAA CUGAUGAGGCCGAAAGGCCGAA ACCAACU
 340 GCACUUC CUGAUGAGGCCGAAAGGCCGAA AGAACCC
 341 UGCACUC CUGAUGAGGCCGAAAGGCCGAA AAGAACC
 350 UAAUGCC CUGAUGAGGCCGAAAGGCCGAA ADGCCAU
 356 UAGGCUU CUGAUGAGGCCGAAAGGCCGAA ADGCCAU
 357 GUAGGCU CUGAUGAGGCCGAAAGGCCGAA AAUGCCA
 363 UGGCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGCCUA
 372 ADGGGAG CUGAUGAGGCCGAAAGGCCGAA ADGCCUU
 375 AUUADGG CUGAUGAGGCCGAAAGGCCGAA AGUADGC
 380 UGUAAAU CUGAUGAGGCCGAAAGGCCGAA ADGGGAG
 383 ACUUGUA CUGAUGAGGCCGAAAGGCCGAA AUUADGG
 385 ATACTUG CUGAUGAGGCCGAAAGGCCGAA AUUADAU
 391 GAGAUCA CUGAUGAGGCCGAAAGGCCGAA ACUUGUA
 396 CGAGUUA CUGAUGAGGCCGAAAGGCCGAA ADCAUAC
 398 AUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGAACAU
 402 AUUUAUG CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
 406 UGAAAUU CUGAUGAGGCCGAAAGGCCGAA ADGGAUU
 410 GGUGUGA CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
 411 UGUGUUG CUGAUGAGGCCGAAAGGCCGAA AAUUUAU
 412 UUGUGUU CUGAUGAGGCCGAAAGGCCGAA AAUUTUA
 421 GUGUGAA CUGAUGAGGCCGAAAGGCCGAA AUUUGGU
 423 UUGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUATUG
 424 AUUUGGU CUGAUGAGGCCGAAAGGCCGAA AAUADUG
 432 UGGUUTA CUGAUGAGGCCGAAAGGCCGAA AUUUGGU
 434 GUUGUUU CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
 446 AUCCAUU CUGAUGAGGCCGAAAGGCCGAA AGUUGUU
 448 UUADGCA CUGAUGAGGCCGAAAGGCCGAA AGAGUUG
 454 GUUADGU CUGAUGAGGCCGAAAGGCCGAA ADGCCAU
 458 UGGAGUA CUGAUGAGGCCGAAAGGCCGAA AGUUAUG
 460 UAUGGAG CUGAUGAGGCCGAAAGGCCGAA AUUAGUA
 463 GACIAUG CUGAUGAGGCCGAAAGGCCGAA AGUAIAG
 467 UCUGGAC CUGAUGAGGCCGAAAGGCCGAA ADGGAGU
 470 CCACUUG CUGAUGAGGCCGAAAGGCCGAA ACUADGG
 489 UUACAUU CUGAUGAGGCCGAAAGGCCGAA AUUUCUA
 490 AUUACUA CUGAUGAGGCCGAAAGGCCGAA AAUUUC
 492 AAAUUCAC CUGAUGAGGCCGAAAGGCCGAA AUUADUU
 495 UUUAAAU CUGAUGAGGCCGAAAGGCCGAA ACTUAAA

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAU A AGAAUU	165	UACAUU A ACTAACG
16	UAGAAU U UGAUAG	169	UUUACU A ACGCUU
17	AAGAAU U GATAAGU	175	UAACGU U UGGCUAA
21	AUUGAU A AGUACC	176	AACGUU U GGCUCAG
25	GATAAGU A CCACUUA	181	UUUGGU A AGGCAGU
31	UACACU U AAAUUA	192	CAGGAGU A CAUACAA
32	ACACUU A AAUUAA	196	GAUACAU A CAAUCAA
36	CUAAAU U UAACUCC	201	AUACAU C AAAUUGA
37	UUAAAU U AACUCCC	206	ADCAAU U GAAGGCG
38.	UAAAUU A ACUCCU	216	ADGGCAU U GUGUUG
42	UUUACU C CCUUGGU	221	AUUGGU U UGUGCAU
46	ACUCGU U GGGUAGA	222	UUGGGU U GUGCAUG
50	CCUUGGU U AGAGAGG	231	UCCAGGU U AUUACAA
51	CUUGGU A GAGAGGG	232	GCAGGU A UUCAAG
67	CAGCAAU U CATUGAG	234	AEGGUAU U ACAAGUA
58	ACCAAU C AUUGAGU	235	UGGUAU A CAGUAG
71	AAUUCAU U GAGUAG	241	UACAGU A GUGAU
76	AUUGAGU A UGAAUAA	247	UAGGAGU A UUUGGCC
81	GUAGAU A AAAGUUA	249	GUGAUU U UGCCCCA
87	UAAAAGU U AGAUAC	250	UGAUAUU U GCCCCA
88	AAAAGGU A GADUACA	256	UGGCCU A AUAAUAA
92	GUAGAU U ACUAAA	259	CCUUAU A AUAAUAU
93	UAGAUU A CAAAUU	262	UAAUAU A AUAAUGU
100	ACAAAU U UGUUUGA	265	UAAUAU A UUGUAGU
101	CAAAAU U GUUUGAC	267	AUAAUAU U GUAGUAA
104	AAUUGU U UGACAAU	270	AUAUUGU A GUAAAAU
105	AUUGGU U GACAAUG	273	UUGUAGU A AAAUCCA
120	AUGAAGU A GCAUUGU	278	GUAAAAU C CAAUUC
125	GUAGCAU U GUAAAAA	283	AUCCAU U UCACAAAC
128	GCAGTGU U AAAAATA	284	UCCAUU U CACACAA
129	CAUUGU A AAAATAA	285	CCAAUU C ACACAA
135	UAAAAAU A ACAUGU	300	UGCCAGU A CUACAAA
143	ACAUUGU A UACTUGU	303	CAGUACU A CAAAAGU
145	AUGGUAU A CGAUAA	316	UGGAGGU U AUAAUG
151	UACTUGAU A AAUUAU	317	GGAGGUU A UAUUAGG
155	GAUAAA U AAUACAU	319	AGGUUAU A UAUUGGG
156	AUAAAAU A AUACAU	321	GUUAAU A UGGGAAA
159	AAUUAU A CAUUAA	338	AUGGAAU U AACACAU
163	AAUACAU U UAACUAA	339	UGGAAU A ACACAU
164	AUACAUU U AACUAC	346	AACACAU U GCUCUCA

350 CAUUGCU C UCAACCU
352 UUGGCUU C AACCUAA
358 UCAACCU A AUGGUCU
364 UAAUGGU C UACTAGA
366 ADGGUCU A CUAGAUG
369 GCGCACU A GAAGACA
379 UGACRAU U GOGAAAU
387 GOGAAAU U AAAUUCU
388 UGAAAAU A AAUUCUC
392 AUAAAAU U COCCAAA
393 UCAAAAU C UCCAAA
395 AAAUUCU C CAAAAA
405 AAAAACU A AGGGAGU
412 AAGUGAU U CRACRAU
413 AGUGAUU C AACAAAG
427 GACCRAU U ATAGGAA
428 ACCRAAU A UAGGAGU
430 CAADRAU A UGAACCA
436 UAGGAAU C AAUUAAC
440 AACCAAU U AUCCGAA
441 AUCAAAU A UCOGAAU
443 CAUUAU C UGAUUA
449 UCOGAAU U ACUOGGA
450 CGGAAUU A CUUGGAU
453 AAUUAUCU U GGAGUUG
458 CUUGGAU U UGAUCUU
459 UGGGAUU U GACGUA
463 AUUUGAU C UTAUACC
465 UUGACCU U AACCCAU
466 UGACCUU A ADCCAA
469 UCUTAAU C CTTAAAU
473 AACCCAU A AACUATA
477 CATAAU U ATAAUCA
478 AUAAAAU A UAAUUA
480 AAUUAU A AUUATA
483 UUACAUU U AAUACCA
484 UAAUAAU A ATAAUCA
487 AAUUAU A UCAACTA
489 UUAAAUU C AACUAGC
494 ADCAACU A GCAAAAC
501 AGCMAAU C AAUGUCA
507 UCRAGGU C ACTAACCA
511 UGUACAU A ACACCAU
519 ACACCU U AGUUAU
520 CACCAAU A GUUAATA
523 CAUUGGU U AAUAAAA
524 AUUAGGU A AAUAAAA

Table 34: RSV (1C) HH Ribozyme Sequence

at. Positi n	HH Ribozyme Sequence
10	AAAUUCU CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
16	CUUAUCA CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
17	AUCUADC CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
21	UGGUACU CUGAUGAGGCCGAAAGGCCGAA AUCAAAU
25	UAAGUGG CUGAUGAGGCCGAAAGGCCGAA ACUUAUC
31	UUAUAUU CUGAUGAGGCCGAAAGGCCGAA AGGGGUA
32	UAAAUUU CUGAUGAGGCCGAAAGGCCGAA AAGGGGU
36	GGAGUUA CUGAUGAGGCCGAAAGGCCGAA AUUUAAG
37	GGGAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUUA
38	AGGGAGU CUGAUGAGGCCGAAAGGCCGAA AAUUUA
42	ACCAAGG CUGAUGAGGCCGAAAGGCCGAA AGUUAAA
46	UCAUACC CUGAUGAGGCCGAAAGGCCGAA AGGGAGU
50	CAUCUCU CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
51	CCACUC CUGAUGAGGCCGAAAGGCCGAA AACCAAG
67	CCCAADG CUGAUGAGGCCGAAAGGCCGAA AUUGCTG
68	ACCUAUU CUGAUGAGGCCGAAAGGCCGAA AUUUGCU
71	CAUACTC CUGAUGAGGCCGAAAGGCCGAA AUUAGAU
76	UUUAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCAAU
81	UAACUUU CUGAUGAGGCCGAAAGGCCGAA AUCAUAC
87	GUAAUCU CUGAUGAGGCCGAAAGGCCGAA ACUUUUA
88	UGUAADC CUGAUGAGGCCGAAAGGCCGAA AACUUUU
92	AUUUGGU CUGAUGAGGCCGAAAGGCCGAA AUCAUAC
93	AAUUUGG CUGAUGAGGCCGAAAGGCCGAA AAUCUAA
100	UCRAAAC CUGAUGAGGCCGAAAGGCCGAA AUUUGU
101	GUCAAAC CUGAUGAGGCCGAAAGGCCGAA AAUUUG
104	AUUGUCA CUGAUGAGGCCGAAAGGCCGAA ACRAAU
105	CAUUGUC CUGAUGAGGCCGAAAGGCCGAA AACRAAU
120	ACAADGC CUGAUGAGGCCGAAAGGCCGAA ACUCAU
125	UUUUAAC CUGAUGAGGCCGAAAGGCCGAA AUUCAAC
128	UUUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAADGC
129	UUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACRAUG
135	AGCRUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUA
143	AUCAGUA CUGAUGAGGCCGAAAGGCCGAA AGCRUGU
145	UUADCG CUGAUGAGGCCGAAAGGCCGAA AUUAGCAU
151	AUUAUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGUA
155	AUUGAUU CUGAUGAGGCCGAAAGGCCGAA AUUADGC
156	AAUGUAU CUGAUGAGGCCGAAAGGCCGAA AUUUAU
159	UAAAADG CUGAUGAGGCCGAAAGGCCGAA AUUAAU
163	UUAGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGUA
164	GUUAGU CUGAUGAGGCCGAAAGGCCGAA AUUAGUA
165	CGUUAGU CUGAUGAGGCCGAAAGGCCGAA AAUUGUA

169 AAAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUAAA
 175 UUAGCCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUA
 176 CUGAGCC CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 181 ACUGCCU CUGAUGAGGCCGAAAGGCCGAA AGGCCAA
 192 UUGUAGG CUGAUGAGGCCGAAAGGCCGAA AUCACTG
 196 UUGAUUG CUGAUGAGGCCGAAAGGCCGAA AUGUUAC
 201 UCAAUUU CUGAUGAGGCCGAAAGGCCGAA AUUGUAU
 206 GCGAUUC CUGAUGAGGCCGAAAGGCCGAA AUUUGAU
 216 CAACAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAU
 221 ADGCACA CUGAUGAGGCCGAAAGGCCGAA ACACAAU
 222 CGCGCAC CUGAUGAGGCCGAAAGGCCGAA AACACAA
 231 UUGUAAU CUGAUGAGGCCGAAAGGCCGAA ACAGGCA
 232 CGUGUAA CUGAUGAGGCCGAAAGGCCGAA AACAGUC
 234 UACTUGU CUGAUGAGGCCGAAAGGCCGAA AUAAACAU
 235 CTACUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAACA
 241 AUADACG CUGAUGAGGCCGAAAGGCCGAA ACTUUGA
 247 GGGCAA CUGAUGAGGCCGAAAGGCCGAA AUCCACUA
 249 UAGGGCA CUGAUGAGGCCGAAAGGCCGAA AUADACAC
 250 UTAGGGC CUGAUGAGGCCGAAAGGCCGAA AUADACUA
 256 UUAUUAU CUGAUGAGGCCGAAAGGCCGAA AGGGCAA
 259 AUAUUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGG
 262 ACRAUAU CUGAUGAGGCCGAAAGGCCGAA AUUADUA
 265 ACUACAA CUGAUGAGGCCGAAAGGCCGAA AUUADUA
 267 UUACTAC CUGAUGAGGCCGAAAGGCCGAA AUUADUA
 270 AUUUAUC CUGAUGAGGCCGAAAGGCCGAA ACUADAU
 273 UGGAUUU CUGAUGAGGCCGAAAGGCCGAA ACTACAA
 278 GAAAUUG CUGAUGAGGCCGAAAGGCCGAA AUUUAUC
 283 GUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUUUGAU
 284 UGUUGUG CUGAUGAGGCCGAAAGGCCGAA AUUUGGA
 285 UUGUUGU CUGAUGAGGCCGAAAGGCCGAA AAUUTGG
 300 UUUGUAG CUGAUGAGGCCGAAAGGCCGAA ACUGGCA
 303 CAUUUUG CUGAUGAGGCCGAAAGGCCGAA AGUACUG
 316 CAUAAAU CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 317 CCAAAAU CUGAUGAGGCCGAAAGGCCGAA AACCUCC
 319 UCCCCAU CUGAUGAGGCCGAAAGGCCGAA AUUACCU
 321 UUCCCAC CUGAUGAGGCCGAAAGGCCGAA AUUAAAC
 338 ADGUGUU CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
 339 AAUGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
 346 UGAGAGC CUGAUGAGGCCGAAAGGCCGAA AUUGUGU
 350 AGGUUGA CUGAUGAGGCCGAAAGGCCGAA AGCAAG
 352 UUAGGUU CUGAUGAGGCCGAAAGGCCGAA AGAGCAA
 358 AGACCAA CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
 364 UCGAGUA CUGAUGAGGCCGAAAGGCCGAA ACCAUUA
 366 CAUCAAG CUGAUGAGGCCGAAAGGCCGAA AGACCAU
 369 UGUCAC CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 379 AUUUCAC CUGAUGAGGCCGAAAGGCCGAA AUUGUCA
 387 AGAUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAC
 388 GAGAUUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUA
 392 UUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUUUAU

393 UUUUGGA CGAUGAGGCCGAAAGCCGAA AAUUA
 395 UUUUUG CGAUGAGGCCGAAAGCCGAA AGAUU
 405 AACACU CGAUGAGGCCGAAAGCCGAA AGUUU
 412 AUUGUUG CGAUGAGGCCGAAAGCCGAA AUACAU
 413 CAUGUU CGAUGAGGCCGAAAGCCGAA AUACAU
 427 UUCAUU CGAUGAGGCCGAAAGCCGAA AUACAU
 428 AUUCAU CGAUGAGGCCGAAAGCCGAA AUUGGU
 430 UGAUCA CGAUGAGGCCGAAAGCCGAA AUADUG
 436 GAAUAU CGAUGAGGCCGAAAGCCGAA AUUCAU
 440 UUCAGU CGAUGAGGCCGAAAGCCGAA AUUGAU
 441 AUUCAG CGAUGAGGCCGAAAGCCGAA AUUGAU
 443 UAAUCA CGAUGAGGCCGAAAGCCGAA AUAAADG
 449 UCCAGU CGAUGAGGCCGAAAGCCGAA AUUCAG
 450 ADCCAG CGAUGAGGCCGAAAGCCGAA AUUCAG
 453 CAAADCC CGAUGAGGCCGAAAGCCGAA AGUAU
 458 AAGACU CGAUGAGGCCGAAAGCCGAA AUCCAAG
 459 UAAAGC CGAUGAGGCCGAAAGCCGAA AUCCAAG
 463 GGAAU CGAUGAGGCCGAAAGCCGAA AUCAA
 465 AUUGAU CGAUGAGGCCGAAAGCCGAA AGACAA
 466 UAUUGAU CGAUGAGGCCGAAAGCCGAA AAGACU
 469 AUUAGU CGAUGAGGCCGAAAGCCGAA AUUAGA
 473 UAUAAU CGAUGAGGCCGAAAGCCGAA AUUGGU
 477 UAAUAU CGAUGAGGCCGAAAGCCGAA AUUADG
 478 UUAADU CGAUGAGGCCGAAAGCCGAA AUUUA
 480 UAUUAU CGAUGAGGCCGAAAGCCGAA AUUADU
 483 UGAAU CGAUGAGGCCGAAAGCCGAA AUUADU
 484 UUGAAU CGAUGAGGCCGAAAGCCGAA AUUADU
 487 UAGUUG CGAUGAGGCCGAAAGCCGAA AUUADU
 489 GCGAGU CGAUGAGGCCGAAAGCCGAA AUUADU
 494 GAUUGC CGAUGAGGCCGAAAGCCGAA AUUADU
 501 UGACAU CGAUGAGGCCGAAAGCCGAA AUUUGU
 507 UGUUAGU CGAUGAGGCCGAAAGCCGAA ACACUGA
 511 AUUGGU CGAUGAGGCCGAAAGCCGAA AGUGACA
 519 AUUACU CGAUGAGGCCGAAAGCCGAA AUUGGU
 520 UAUUAC CGAUGAGGCCGAAAGCCGAA AUUGGU
 523 UUAAAU CGAUGAGGCCGAAAGCCGAA ACTUADG
 524 UUUAU CGAUGAGGCCGAAAGCCGAA AACUAAU

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GCGAAAU A CAAAGAU	217	GGUAUUGU U AUUAGCG
21	GAUCCGU C UUAGCRA	218	GTAAUGU A UADGCGA
23	UGGCUUJ U AGCNAAG	220	ADGUUAU A UGGGAGG
24	GCGCJUU A GCAAGU	229	GCGADGU C TAGGTTA
32	GCPAAAGU C AAGUUGA	231	GAUUCUJ A GGUUAGG
37	GUCAAGU U GAUAGAU	235	UCAAGGU J AGGAAGA
45	GAAGAU A CACUCAA	236	CUAGGUU A GGAAGAG
50	AUACACU C AACNAAG	254	ACACCAU A AAAAUAC
60	CAAGAU C AACUUCU	260	AAAAAUU A CCTAGAG
65	AUCACAU U CGUCUAU	263	AAACACU C AGAGAGG
66	UCAACAU C UGUCUGC	277	GCGGGAU A UCAUGUA
70	CUUCUGU C ADCCAGC	279	GCGAAAU C ADGUAAA
73	CGUCUAU C CAGCAA	284	ADCAUGU A AAAGCAA
82	AGCAAAU A CACCAUC	299	ADGGAGU A GALGUAA
89	ACACCAU C CAACGGA	305	UAGAGGU A ACACAC
108	AGGAGAU A GTAUUGA	315	AAACAUJ C GUCAGA
111	AGAUUAGU A UUGUATAC	318	ACACCGU C AAGACAU
113	AUAGUAU U GAAUACUC	326	AAGACAU U AACGGAA
117	UATUGAU A CCUCAA	327	AGACAUU A ADGGAAA
120	UGAUUACU C CUAUUA	346	ADGAAAU U UGAGGUG
123	UACTUCU A AUUADGA	347	UGAAAUU U GAAGGUG
126	UCCUAUU U ADGADGU	355	GAAGGUGU U AACRUG
127	CCUAAAU A UGADGUG	356	AAGGUGU A ACATUGG
146	AACACAU C AAUAGU	361	UUAACAU U GGCAAGC
150	CAUCAAU A AGUUAAG	370	GCAAGCU U AACACAU
154	AADAAGU U ADGUGGC	371	CAAGCU A ACACACU
155	ADTAAGOU A UGUGGCA	383	CGUAAAU U CAAUCA
166	GCGADGU U ADUADC	384	UGAAAUU C AAACUCA
167	GCAOGGU A UUAADCA	389	UCCUAUU C AACAUUG
169	ADGUUAU U AACACAU	395	UCAACAU U GAGAUAG
170	UGUUAUU A ADCACAG	401	UUGAGAU A GAACUCA
173	UATUUAU C ACAGAAC	406	AUAGAAAU C UAGAAA
186	AGAUUCU A ADCAUAA	408	AGAACUJ A GAAAUC
189	UGCTUAU C ADAAAUU	415	AGAAAAU C CUAACRAA
192	UAAUCAU A AACUAC	418	AAAUCU A CAAAAAA
196	CAUAAAU U CACUGGG	431	AAAUGCU A AAAGAAA
197	AUAAAAU C ACUGGGU	449	GAGAGGU A GCUCCAG
205	ACUGGGU U AAUAGGU	453	GGUAGCU C CAGAAUA
206	CGUGGUU A AUAGGU	460	CCAGAAU A CAGGCAU
209	GGUUAUU A GGUAUGU	472	CAGACAU C UCCUGAU
213	AAUAGGU A UGUAUUA	474	UGACUCU C CGAGTUG

480	UCCUGAU U GUGGGAU	696	UUUTGGU A UAGCACA
491	GGAUJAU A AUAAUAU	698	UUGGJAU A GCACJAU
494	UGAUAU A UUAUGUA	706	GCACAU C UUCJACC
496	AUAADAU U AUGUAUA	708	ACAAUCU U CUACCA
497	UAAAUU A UGUAIAG	709	CAUUCU C UACCA
501	AUUAUGU A UAGCAGC	711	AUCUUCU A CCAGAGG
503	UADGUAU A GCAGCAU	726	UGGCAGU A GAGUJUG
511	GCAGCAU U AGUAUUA	731	GUAGAGU U GAAGGGA
512	CAGCAU A GUAAUAA	740	AAGGGAU U UUUGCAG
515	CAUAGU A AUAACUA	741	AGGGAUU U UUGCAGG
518	UAGUAAU A ACUAAA	742	GGGAGUU U UGCAGGA
522	AUAACU A AAUAGC	743	GGAUUUU U GCAGGAU
526	ACUAAA U AGCAGCA	751	GCAGGAU U GUUUAUG
527	CUAAAUU A GCAGCAG	754	GGAUJUGU U UADGAAU
544	GACAGAU C UGGCUU	755	GAUOGUU U AUGAADG
549	AUCUGGU C UUACAGC	756	AUUGUUU A UGAADGC
551	CGUGCU U ACAGCG	766	AAUGCCU A UGGUGCA
552	UGGUCUU A CAGCGU	787	GUAGUGU U ACGGUGG
563	CGUGAU U AGGAGAG	788	UGAUGUU A CGGUGGG
564	CGUGAUU A GGAGAGC	800	GGGGAGU C UUAGCAA
573	GAGAGCU A AUAADGU	802	GGAGCU U AGCAAA
576	AGCTUAU A ADGCCU	803	GAGCUU A GCIAAAU
581	AUAADGU C CUAAAAA	811	GCIAAAAU C AGUAAA
584	AUGUCCU A AAAAAG	815	AACAGC U AAAAADA
603	GAACGU U ACUAGG	816	AUCAGUU A AAAAADA
604	AAACGUU A CAAGGC	822	UAAAAAU A UUADGUU
613	AAAGGUU U ACUACCC	824	AAAUAU U AUGUUAG
614	AAGGUU A CUACCCA	825	AAAUAU U UGUUAGG
617	GCUUCAU A CCCAAGG	829	AUUAUGU U AGGACAU
629	AGGACAU A GCACACA	830	UUAUGUU A GGACAU
640	AACAGCU U CUAGUA	840	ACAGCU A GUGUGCA
641	ACAGCUU C UUAGAG	866	AAACAGU U GUUGAGG
643	AGCUUCU A UGAAGUG	869	AAUGUUGU U GAGGUUU
652	GAAGGUU U UGAAAAA	875	UUGAGGU U UUAGAAU
653	AAGUGUU U GAAAAC	876	UGAGGUU U AUGAUA
663	AAAACAU C CCCACUU	877	GAGGUU A UGAUAU
670	CCCCACU U UUAGAU	883	UADGAAU A UGCCAA
671	CCCCAUU U AUAGAUG	895	CAAAAU U GGGUGGU
672	CCCCUUAU A UAGAGU	913	GCAGGAU U CUACCAU
674	AUUAUUAU A GAUGUU	914	CAGGAU C UACCA
680	UAGAGGU U UUUGUOC	916	GGAUUCU A CCACAU
681	AGAUGUU U UUGUOCA	921	CUACCAU A UAUUGAA
682	GAUGUUU U UGUOCAU	923	ACCAAU A UUGAAC
683	AUGUUUU U GUOCADU	925	CAUAAU U GAACAAC
686	UUUUUGU U CAUUUG	943	AAAGCAU C AUUAUJA
687	UUUUGUU C AUUUTGG	946	GCAUCAU U AUUAUCU
690	UGUUCAU U UGGCAU	947	CAUCAU A UUAUCUU
691	GUUCAU U UGGUUA	949	UCAUUAU U AUCUUUG
692	UUCAUU U GGUAUAG	950	CAUAAU A UCUUUGA

952	UUAUUAU C UUUGACU
954	AUUAUCU U UGACUCA
955	UUAUCCU U GACUCAA
960	UUUGACU C AAUUCC
964	ACUCAAU U UCCUCAC
965	CUCAAAU U CCUCACU
966	UCAAUUU C CCACAUU
969	AUUUCU C ACUUCU
973	CCUCACU U CCUCAGU
974	CUCAUU C UCCAGUG
976	CAUUCU C CAGUGUA
983	CCAGUGU A GUADUAG
986	GUGUAGU A UUAGGCA
988	GUAGUAU U AGGCRAU
989	UAGUAU A GGCAADG
1007	CUGGCCU A GGCAUAA
1013	UAGGCCU A AUGGGAG
1024	GGAGAGU A CAGAGGU
1032	CAGAGGU A CACCGAG
1044	GAGGAU C AAGAUCU
1050	UCAAGAU C UAAUAGA
1052	AAGAUCU A UAGGAGG
1054	GAUCAAU A UGAGCAG
1072	AAGCCAU A UGGUGAA
1085	AACAAUCU C AAAGAAA
1103	GUGUGAU U AACUACA
1104	UGUGAUU A ACUACAG
1108	AUUAUCU A CAGUGUA
1115	ACAGUGU A CUAGACU
1118	GUGUACU A GACUUGA
1123	CUAGACU U GACACCA
1139	AAGAACU A GAGGCUA
1146	AGAGGCCU A UCAAACA
1148	AGGCAAU C AAACAU
1155	CAAAACAU C AGCTTAA
1160	AUCAGCU U AADCCAA
1161	UCAGCUU A ADCCCAA
1164	GCUUAAU C CAAAAGA
1173	AAAAGAU A ADGAUGU
1181	ADGAUGU A GACCUUU
1187	UAGAGCU U UGAGUUA
1188	AGAGCCU U GAGUUA
1193	UUUGAGU U AAUAAA
1194	UUGAGGU A AUAAAAAA

Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
21	UCCGUAA CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
23	CUUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCCA
24	ACUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGAGCC
32	UCAACUU CUGAUGAGGCCGAAAGGCCGAA ACUUUGC
37	AUCAUUC CUGAUGAGGCCGAAAGGCCGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
50	CUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGUUAU
60	AGAAGUU CUGAUGAGGCCGAAAGGCCGAA AUUUUG
65	ADGACAG CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
66	GAGGACA CUGAUGAGGCCGAAAGGCCGAA XAGTUGA
70	GCUGGAU CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
73	UUUGCUG CUGAUGAGGCCGAAAGGCCGAA ADGACAG
82	GAUGGUG CUGAUGAGGCCGAAAGGCCGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCCGAAAGGCCGAA ADGGGUU
108	UCAAAAC CUGAUGAGGCCGAAAGGCCGAA ADUCCU
111	GUAUCAA CUGAUGAGGCCGAAAGGCCGAA ACTAUU
113	GAGUAUC CUGAUGAGGCCGAAAGGCCGAA AUUACU
117	UUAGGAG CUGAUGAGGCCGAAAGGCCGAA AUCAATA
120	UAAUUAG CUGAUGAGGCCGAAAGGCCGAA AGUAUCA
123	UCAAAAU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUAGGA
127	CACAUCA CUGAUGAGGCCGAAAGGCCGAA AADUAGG
146	ACUUAU CUGAUGAGGCCGAAAGGCCGAA ADGGGUU
150	CAAAACU CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
154	GCACACU CUGAUGAGGCCGAAAGGCCGAA ACUUAUU
155	UCCACAC CUGAUGAGGCCGAAAGGCCGAA AACUUAU
166	GAUUAU CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
167	UGAUUAA CUGAUGAGGCCGAAAGGCCGAA AACAGGC
169	UGUGAUU CUGAUGAGGCCGAAAGGCCGAA AUUACAU
170	CUGUGAU CUGAUGAGGCCGAAAGGCCGAA AACUACA
173	CUUUGU CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
186	UUAUUAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
189	AAUUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGCA
192	GUGAUU CUGAUGAGGCCGAAAGGCCGAA ADGAAUA
196	CCCCAGU CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
197	ACCCAGU CUGAUGAGGCCGAAAGGCCGAA AUUUAU
205	ACCUAAU CUGAUGAGGCCGAAAGGCCGAA ACCCAGU
206	UACCUAU CUGAUGAGGCCGAAAGGCCGAA AACCCAG
209	ACAUACC CUGAUGAGGCCGAAAGGCCGAA AUUAAAC
213	UAAUACA CUGAUGAGGCCGAAAGGCCGAA ACCUAAU

217 CGCAUAU CUGAUGAGGCCGAAAGGCCGAA ACATACC
 218 UOGCAUA CUGAUGAGGCCGAAAGGCCGAA AACAUAC
 220 CADCGCA CUGAUGAGGCCGAAAGGCCGAA AATAACAU
 229 UAACTUA CUGAUGAGGCCGAAAGGCCGAA ACAGUCGC
 231 CCUAAACC CUGAUGAGGCCGAAAGGCCGAA AGACAUU
 235 UCUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCUAGA
 236 CUCUCCC CUGAUGAGGCCGAAAGGCCGAA AACCUAG
 254 GUAUUUU CUGAUGAGGCCGAAAGGCCGAA AUGUGGU
 260 CUCUGAG CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
 263 CAUCUCU CUGAUGAGGCCGAAAGGCCGAA AGUAUUU
 277 UACAGGA CUGAUGAGGCCGAAAGGCCGAA ADGCCGC
 279 UUUCAU CUGAUGAGGCCGAAAGGCCGAA AATAUCC
 284 UUGCUCU CUGAUGAGGCCGAAAGGCCGAA ACAGAU
 299 UUACADC CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
 305 GUUUGU CUGAUGAGGCCGAAAGGCCGAA ACAGUUA
 315 UCUUGAC CUGAUGAGGCCGAAAGGCCGAA AUGUGU
 318 AUGUCUU CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
 326 UUCCUU CUGAUGAGGCCGAAAGGCCGAA AUGUCUU
 327 UUUCUU CUGAUGAGGCCGAAAGGCCGAA AUGUCU
 346 CACUCA CUGAUGAGGCCGAAAGGCCGAA AUUCAU
 347 ACACUUC CUGAUGAGGCCGAAAGGCCGAA AUUUCA
 355 CAAGGUU CUGAUGAGGCCGAAAGGCCGAA ACACUUC
 356 CCAAGGU CUGAUGAGGCCGAAAGGCCGAA AACACU
 361 GCUGGCC CUGAUGAGGCCGAAAGGCCGAA AUGUUA
 370 AGUUGUU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
 371 CAGUUGU CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
 383 UGAUUGG CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
 384 UUGAUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCA
 389 CAAGGUU CUGAUGAGGCCGAAAGGCCGAA AUUUGAA
 395 CUACUCU CUGAUGAGGCCGAAAGGCCGAA AUGUGUA
 401 UAGAUUC CUGAUGAGGCCGAAAGGCCGAA AUUCAA
 406 UUUCUA CUGAUGAGGCCGAAAGGCCGAA AUUCAU
 408 GAUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAUUCU
 415 UUUGUAG CUGAUGAGGCCGAAAGGCCGAA AUUUCU
 418 UUUCUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUU
 431 UUUCUU CUGAUGAGGCCGAAAGGCCGAA AGCAUUU
 449 CGGGAGC CUGAUGAGGCCGAAAGGCCGAA ACCUCUC
 453 UAUUCUG CUGAUGAGGCCGAAAGGCCGAA AGCUACC
 460 AUGCCUG CUGAUGAGGCCGAAAGGCCGAA AUUCUG
 472 AUCAAGGA CUGAUGAGGCCGAAAGGCCGAA AGUCADG
 474 CAUCAG CUGAUGAGGCCGAAAGGCCGAA AGAGUCA
 480 AUCCCCAC CUGAUGAGGCCGAAAGGCCGAA ADCRAGGA
 491 AATAATAU CUGAUGAGGCCGAAAGGCCGAA AUCAUCC
 494 UACATAA CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
 496 UAUACAU CUGAUGAGGCCGAAAGGCCGAA AUAUUAU
 497 CUUACA CUGAUGAGGCCGAAAGGCCGAA AUUAUUA
 501 GCUGCWA CUGAUGAGGCCGAAAGGCCGAA ACATAAU
 503 ADGCCUGC CUGAUGAGGCCGAAAGGCCGAA AUACAU
 511 UAUUACU CUGAUGAGGCCGAAAGGCCGAA AUGCUGC

512 UUAUUAC CUGAUGAGGCCGAAAGGCCGAA AAUCUG
 515 UAGUUAU CUGAUGAGGCCGAAAGGCCGAA ACUAAG
 518 AUUUAU G CUGAUGAGGCCGAAAGGCCGAA AUUACTA
 522 GCTAAUU CUGAUGAGGCCGAAAGGCCGAA AGUUAUU
 526 UGCUGCU CUGAUGAGGCCGAAAGGCCGAA AUUAGU
 527 CGCUGC CUGAUGAGGCCGAAAGGCCGAA AUUAG
 544 AAGACCA CUGAUGAGGCCGAAAGGCCGAA AUUCGUC
 549 GCGUUA CUGAUGAGGCCGAAAGGCCGAA ACCAGAU
 551 CGGCGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
 552 ACGCGUG CUGAUGAGGCCGAAAGGCCGAA AAGACCA
 563 CUCUCU CUGAUGAGGCCGAAAGGCCGAA AUCAGG
 564 GCGUCU CUGAUGAGGCCGAAAGGCCGAA AUCAUG
 573 ACAUUAU CUGAUGAGGCCGAAAGGCCGAA AGCUCUC
 576 AGGACAU CUGAUGAGGCCGAAAGGCCGAA AUUAGCU
 581 UUUUUG CUGAUGAGGCCGAAAGGCCGAA ACAUUAU
 584 CAUUUUU CUGAUGAGGCCGAAAGGCCGAA AGGACAU
 603 CCUUCGU CUGAUGAGGCCGAAAGGCCGAA ACGUUCC
 604 GCGUUGG CUGAUGAGGCCGAAAGGCCGAA AACGUUU
 613 GGGUAG CUGAUGAGGCCGAAAGGCCGAA AGCCUUU
 614 UGGGUAG CUGAUGAGGCCGAAAGGCCGAA AAGCCUU
 617 CCUCUGG CUGAUGAGGCCGAAAGGCCGAA AGUUAAGC
 629 UGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUGUCCU
 640 UUCUAG CUGAUGAGGCCGAAAGGCCGAA AGCUUU
 641 CUUCUA CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
 643 CACUUC CUGAUGAGGCCGAAAGGCCGAA AGAACGU
 652 UUUUUCU CUGAUGAGGCCGAAAGGCCGAA ACACUUC
 653 GGUUUC CUGAUGAGGCCGAAAGGCCGAA AACACUU
 663 AAGUGGG CUGAUGAGGCCGAAAGGCCGAA AUGUUUU
 670 AUUUAUA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 671 CAUCUA CUGAUGAGGCCGAAAGGCCGAA AAGUGGG
 672 ACACUUA CUGAUGAGGCCGAAAGGCCGAA AAAGUGG
 674 AAACAU CUGAUGAGGCCGAAAGGCCGAA AUAAAAGU
 680 GAACAA CUGAUGAGGCCGAAAGGCCGAA ACACUUA
 681 UGAACAA CUGAUGAGGCCGAAAGGCCGAA AACACU
 682 ADGAACA CUGAUGAGGCCGAAAGGCCGAA AAACACU
 683 AAAGAC CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 686 CAAAAG CUGAUGAGGCCGAAAGGCCGAA ACACAAA
 687 CCAAAAT CUGAUGAGGCCGAAAGGCCGAA AACAAA
 690 ADACCAA CUGAUGAGGCCGAAAGGCCGAA ADGAACA
 691 UADACCA CUGAUGAGGCCGAAAGGCCGAA AAUDGAAC
 692 CCAACCC CUGAUGAGGCCGAAAGGCCGAA AAADGAA
 696 UGUGCUA CUGAUGAGGCCGAAAGGCCGAA ACCAAAA
 698 AUUGUGC CUGAUGAGGCCGAAAGGCCGAA AUACCAA
 706 GGJAGAA CUGAUGAGGCCGAAAGGCCGAA AUUGUGC
 708 CGGGTAG CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
 709 UCUGGUA CUGAUGAGGCCGAAAGGCCGAA AAGAUUG
 711 CCUCUGG CUGAUGAGGCCGAAAGGCCGAA AGAACAU
 726 UCAACUC CUGAUGAGGCCGAAAGGCCGAA ACUGCCA
 731 UCCCCUC CUGAUGAGGCCGAAAGGCCGAA ACUCUAC

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740 CUGAAA CGAUGAGGCCGAAAGGCCGAA AUCCCCU
 741 CGUCGA CGAUGAGGCCGAAAGGCCGAA AADCCCU
 742 UCCUGCA CGAUGAGGCCGAAAGGCCGAA AAAUCC
 743 AUCCUGC CGAUGAGGCCGAAAGGCCGAA AAAAUCC
 751 CAUAAAC CGAUGAGGCCGAAAGGCCGAA AUCCUGC
 754 AUCCAU CGAUGAGGCCGAAAGGCCGAA ACACAUCC
 755 CAUCAU CGAUGAGGCCGAAAGGCCGAA AACAUAC
 756 GCAUCA CGAUGAGGCCGAAAGGCCGAA AAACAU
 766 UGCACCA CGAUGAGGCCGAAAGGCCGAA AGGCCAU
 787 CCACCGU CGAUGAGGCCGAAAGGCCGAA ACACUAC
 788 CCCACCG CGAUGAGGCCGAAAGGCCGAA AACACCA
 800 UUGCATA CGAUGAGGCCGAAAGGCCGAA ACUCCCC
 802 UUUGCGU CGAUGAGGCCGAAAGGCCGAA AGACUCC
 803 AUUUUGC CGAUGAGGCCGAAAGGCCGAA XAGACUC
 811 UUUACAU CGAUGAGGCCGAAAGGCCGAA AUUUUGC
 815 UAUUUU CGAUGAGGCCGAAAGGCCGAA ACUGAU
 816 AUAUUU CGAUGAGGCCGAAAGGCCGAA AACUGAU
 822 AACAUAA CGAUGAGGCCGAAAGGCCGAA AUUUUUA
 824 CUACAU CGAUGAGGCCGAAAGGCCGAA AUAUUUU
 825 CCUACAU CGAUGAGGCCGAAAGGCCGAA AAUAUUU
 829 ADGCCCCU CGAUGAGGCCGAAAGGCCGAA ACACAU
 830 CADGCC CGAUGAGGCCGAAAGGCCGAA AACAUAA
 840 UGGCACAC CGAUGAGGCCGAAAGGCCGAA AGCACGU
 866 CCUCAAC CGAUGAGGCCGAAAGGCCGAA ACUUGUU
 869 AAACCC CGAUGAGGCCGAAAGGCCGAA ACACAU
 875 AUCCAU CGAUGAGGCCGAAAGGCCGAA ACCUCAA
 876 UAUCAU CGAUGAGGCCGAAAGGCCGAA AACCUCA
 877 AUAUCA CGAUGAGGCCGAAAGGCCGAA AAACCU
 883 UUGGCGA CGAUGAGGCCGAAAGGCCGAA AUUCAU
 895 ACCACCC CGAUGAGGCCGAAAGGCCGAA AUUUUUG
 913 AUUGGAG CGAUGAGGCCGAAAGGCCGAA AUCCUGC
 914 UAUGGUA CGAUGAGGCCGAAAGGCCGAA AUCCCG
 916 UAUAUGG CGAUGAGGCCGAAAGGCCGAA AGAACCC
 921 UUCAUAA CGAUGAGGCCGAAAGGCCGAA AUUGGAG
 923 UGUUCAU CGAUGAGGCCGAAAGGCCGAA AUAUUGG
 925 GUUGUUC CGAUGAGGCCGAAAGGCCGAA AUAUUGG
 943 UAUAUAU CGAUGAGGCCGAAAGGCCGAA AUUGCUUU
 946 AGAUAAU CGAUGAGGCCGAAAGGCCGAA AUGAUGC
 947 AAGAUAA CGAUGAGGCCGAAAGGCCGAA AUUGAUG
 949 CAAGAU CGAUGAGGCCGAAAGGCCGAA AUUAUGA
 950 UCAAGAA CGAUGAGGCCGAAAGGCCGAA AUUAUG
 952 AGUCAAA CGAUGAGGCCGAAAGGCCGAA AUUAUAA
 954 UGAGUCA CGAUGAGGCCGAAAGGCCGAA AGAUAAU
 955 UUGAGUC CGAUGAGGCCGAAAGGCCGAA AAGAUAA
 960 GGAAAUU CGAUGAGGCCGAAAGGCCGAA AGUCAAA
 964 GUGAGGA CGAUGAGGCCGAAAGGCCGAA AUUGAGU
 965 AGUGAGG CGAUGAGGCCGAAAGGCCGAA AUUGAG
 966 AAGUGAG CGAUGAGGCCGAAAGGCCGAA AAUUGGA
 969 GAGAAGU CGAUGAGGCCGAAAGGCCGAA AGGAAAU

973 ACTGGAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGG
 974 CACTGGA CUGAUGAGGCCGAAAGGCCGAA AAGTGAG
 976 UACACUG CUGAUGAGGCCGAAAGGCCGAA AGAACGG
 983 CTAAATAC CUGAUGAGGCCGAAAGGCCGAA ACACCGG
 986 UGCCTAA CUGAUGAGGCCGAAAGGCCGAA ACTACAC
 988 AUUGCCU CUGAUGAGGCCGAAAGGCCGAA AUACAC
 989 CAUUGCC CUGAUGAGGCCGAAAGGCCGAA AAUACUA
 1007 UUAUGCC CUGAUGAGGCCGAAAGGCCGAA AGCCCAG
 1013 CUCCCAU CUGAUGAGGCCGAAAGGCCGAA AUGCCUA
 1024 ACCUCUG CUGAUGAGGCCGAAAGGCCGAA ACTCCAC
 1032 CUCGGUG CUGAUGAGGCCGAAAGGCCGAA ACCJCG
 1044 AGADCUU CUGAUGAGGCCGAAAGGCCGAA AUCCCAC
 1050 UCAUATA CUGAUGAGGCCGAAAGGCCGAA ACCJUGA
 1052 CAUCATA CUGAUGAGGCCGAAAGGCCGAA AGADCUU
 1054 UGCAUCA CUGAUGAGGCCGAAAGGCCGAA AUAGAC
 1072 UUCAGCA CUGAUGAGGCCGAAAGGCCGAA AUGCCUU
 1085 UUUCUU CUGAUGAGGCCGAAAGGCCGAA AGUUGUU
 1103 UGUAGUU CUGAUGAGGCCGAAAGGCCGAA AUCCACAC
 1104 CGUAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCACA
 1108 UACACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAAU
 1115 AGUCUAG CUGAUGAGGCCGAAAGGCCGAA ACACCGU
 1118 UCAAGUC CUGAUGAGGCCGAAAGGCCGAA AGAACAC
 1123 UGCUGUC CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
 1139 UAGCCUC CUGAUGAGGCCGAAAGGCCGAA AGUUCUU
 1146 UGUUUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUCU
 1148 GAUGUUU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
 1155 UUAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGUUUG
 1160 UUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
 1161 UUUGGGAU CUGAUGAGGCCGAAAGGCCGAA AAGCTGA
 1154 UCUUUUG CUGAUGAGGCCGAAAGGCCGAA AUUAGC
 1173 ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
 1181 AAAGCUC CUGAUGAGGCCGAAAGGCCGAA ACACUAI
 1187 UAACUCA CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
 1188 UUAACUC CUGAUGAGGCCGAAAGGCCGAA AAGCUCU
 1193 UUUUAUU CUGAUGAGGCCGAAAGGCCGAA ACUCUAA
 1194 UUUUUAU CUGAUGAGGCCGAAAGGCCGAA AACTCAA

Table 37: RSV (1B) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
70	CUGUAC AGAA GCUU ACCAGAAAACCAACGUUCCUUGA	AAAACU GAU GAAUCAG
91	CGAGAAC AGAA GCUU ACCAGAAAACCAACGUUCCUUGA	UGAGCC GUU GCAACUG
472	CAGGUCC AGAA GCUU ACCAGAAAACCAACGUUCCUUGA	UAUCCA GAU GAAACCUU

Table 38: RSV (N) IIP Ribozyme/Substrate Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
476	AUCCCA AGA ACCAGAGAAAACACGGUGGGUUCGUUUCGUU	CUCUCCU GAA UGUGGAAU
540	AGAACCGG AGA ACCAGAGAAAACACGGUGGGUUCGUUUCGUU	GCCCCCA GAA CGGGCUU
554	CUNAUUCG AGA ACCAGAGAAAACACGGUGGGUUCGUUUCGUU	UCUUAUA GGC GUAGUUG
636	UUCUAGGA AGA ACCAGAGAAAACACGGUGGGUUCGUUUCGUU	GCACCA GCU UCUUAUA
998	CCUAGGCC AGA ACCAGAGAAAACACGGUGGGUUCGUUUCGUU	CAUGCU GCU GGCCUAGG
1156	UUCGAAU AGA GAUGUU ACCAGAGAAAACACGGUGGGUUCGUU	AUAGUAU GCU UAUUCCAU

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Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	89
(GGU) ₃ GGT	T [0.50/0.33]	[0.1/0.02]	15 m	78
(GGU) ₃ GGT	S [0.25/0.17]	[0.1/0.02]	15 m	81
C ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-O-methyl coupling. S = 5-S-Ethyltetrazole, T = tetrazole activator. A is 5' -ucu ccA UCU GAU GAG GCC GAA AGG CCG AAA Auc ccu -3' where lowercase represents 2'-O-methylnucleotides.

Table 40: Base Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU) ₄	NH ₄ OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU) ₄	NH ₄ OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C ₉ U	NH ₄ OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH ₄ OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

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Table 41: 2'-O-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
A ₉ T	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
(GGU) ₄	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C ₁₀	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U ₁₀	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU
-3'.

**Table 42 : NMR Data for UC Dimers containing
Phosphorothioate Linkage**

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

*Table 43: NMR Data for 15-mer RNA containing
Phosphorothioate Linkages*

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
<hr/>					
3532	2'-O-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-O-Me	2 x 4 s	13.8	2 x 300 s	99.8
3682	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	k (min^{-1})*
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reeding, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

The equation describes the extent of ribozyme processing in the presence of ongoing transcription (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (\pm range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH ₂ -U	6.5	120	180
4	U7 = 2'=CH ₂ -U	8	280	350
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130
6	U4 = 2'=CF ₂ -U	5	320	640
7	U7 = 2'=CF ₂ -U	4	220	550
8	U4 & U7 = 2'=CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

CLAIMSWhat is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.
2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37.
3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.
4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora VS* RNA or RNaseP RNA motif.
5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.
6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.
7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.
8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.
9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.
- 30 10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

11. The cell of claim 10, wherein said cell is a human cell.
12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.
5
13. A mammalian cell including an expression vector of claim 12.
14. The cell of claim 13, wherein said cell is a human cell.
15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.
10
16. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an expression vector of claim 12.
17. The method of claims 15 or 16, wherein said patient is a human.
15
18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infarction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.
20
19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
25
20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
30

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
- 5 22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.
23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.
- 10 24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
- 15 25. An oligonucleotide comprising a moiety having the formula:

wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.
- 20 26. An oligonucleotide comprising a 3'-amido or peptido group.
27. An oligonucleotide comprising a 5'-amido or peptido group.
- 25 28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.
- 30 29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.

30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarboxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.

5

31. Method for the synthesis of a nucleoside 5' or a 3'-dihalo-methylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.

10

32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.

33. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.

15

34. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.

20

35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH₄OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.

25

36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine-hydrogen fluoride (aHF-TEA) trimethylamine or disopropylethylamine at between 60 °C-70 °C for 0.25-24 h.

30

37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPCC column is an anion exchange chromatography column.

38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.

5

39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 300 seconds.

10

40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-S-ethyltetrazole or 5-S-methyltetrazole prior to sulfurization.

15

41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.

42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phthaloyl group.

20

43. The method of claim 42 wherein the said nucleoside lacks a base.

44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.

25

45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.

46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.

30

47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions.

48. The method of claim 57 wherein said ($\text{BF}_3 \cdot \text{OEt}_2$) is provided in acetonitrile.

49. One or more vectors comprising

5 a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;

10 and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other nucleic acid sequences encoding RNA which is cleaved by said first ribozyme to release said second ribozyme from RNA encoded by said vector;

15 20 wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which reduces release of said second ribozyme by more than 20%.

50. Cell comprising the vector of claim 49.

51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.

25 52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.

30 53. The RNA molecule of claim 51, wherein said molecule is transcribed by a type 2 pol III promoter system.

54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecule of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.
56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.
- 5 57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.
58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.
- 10 59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.
60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.
- 15 61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.
62. DNA vector encoding the RNA molecule of claim 51
63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.
- 20 64. RNA vector encoding the RNA molecule of claim 51.
65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.
66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.
- 25 67. Cell comprising the vector of claim 62.
68. Cell comprising the vector of claim 53.
69. Cell comprising the RNA of claim 51.

70. Method to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.
- 5 71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.
72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.
- 10 73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) in trans.
- 15 74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.
75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 4, and m is 1 - 20.
- 20 76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.
77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
- 25 78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
79. The ribozyme of claim 73 having the structure of Fig. 73.
80. The ribozyme of claim 73 having the structure of Fig. 74.
- 30 81. A cell including the ribozyme of any of claims 73-80.

82. An expression vector comprising nucleic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozyme within a cell.
83. A cell including an expression vector of claim 82.
- 5 84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:

contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair in vivo, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.

10 85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.
- 15 86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.

20 87. The method of claim 84, wherein said nucleic acid molecule is DNA or RNA.

88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.

25 89. The method of claim 88, wherein said mutagen is nitrous acid.

90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

30 91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;

providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid

5 molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

10 and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.

93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

15 providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter

20 region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

25 and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.

94. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

30 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

5 and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

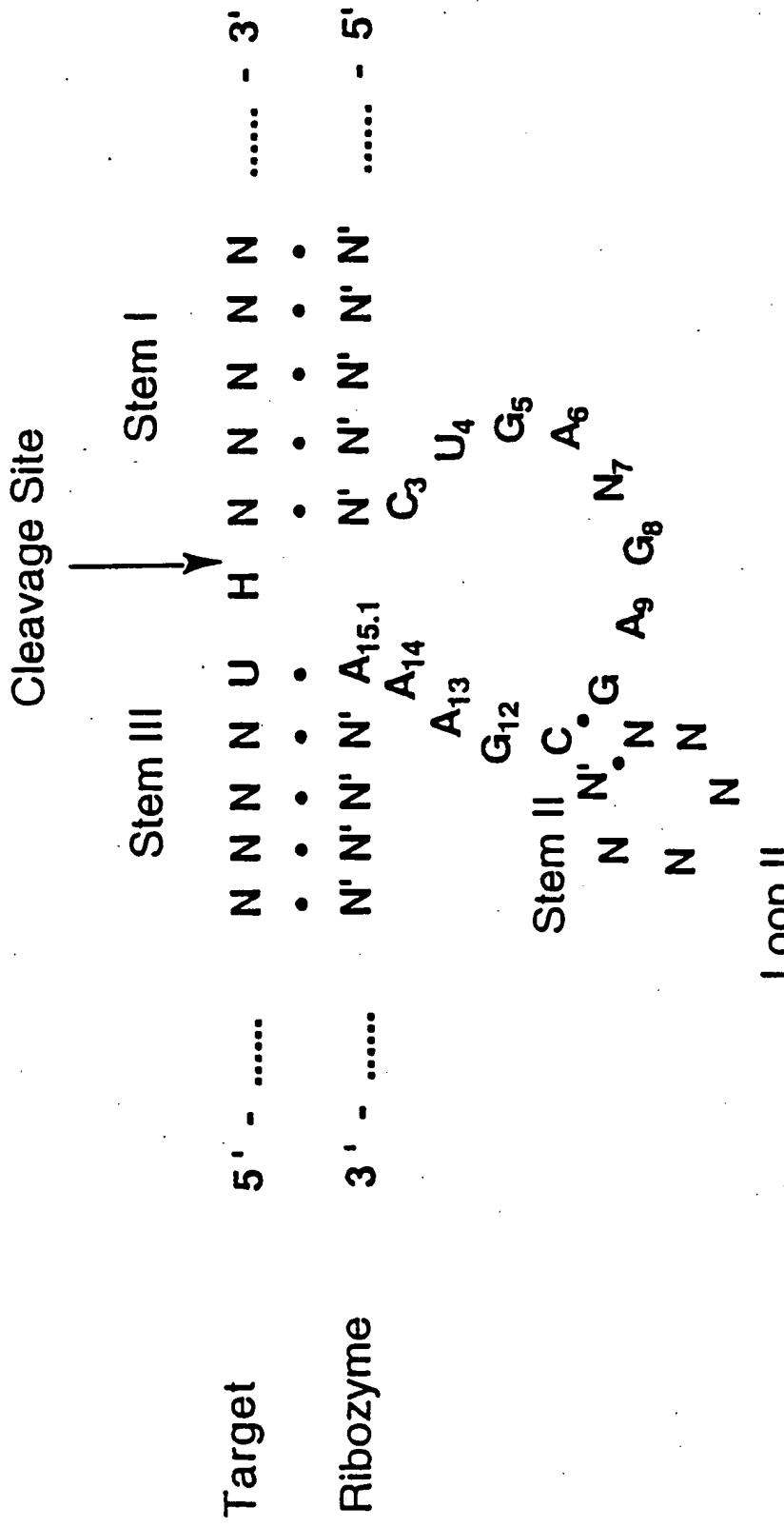
10 95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

15 96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

20 97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

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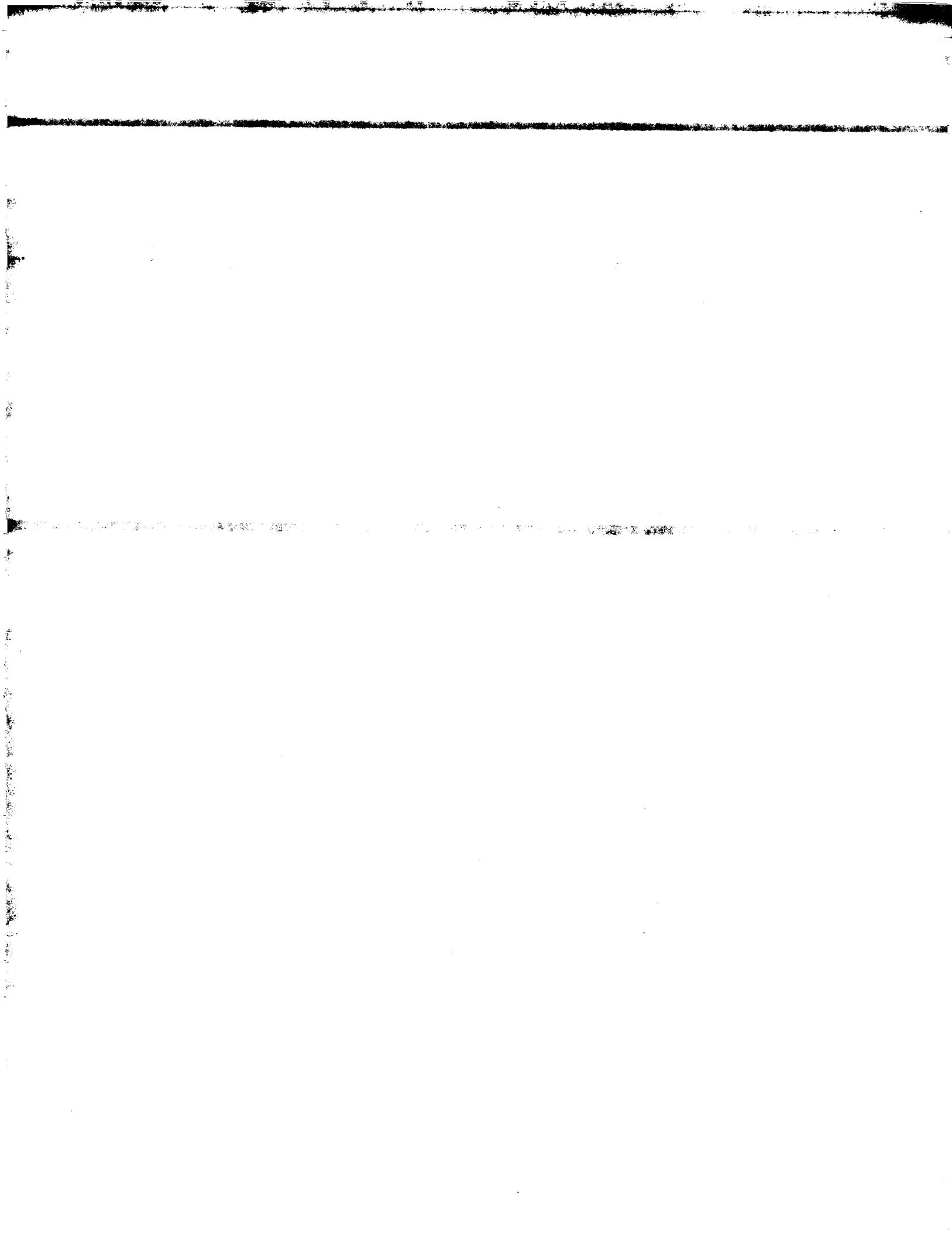
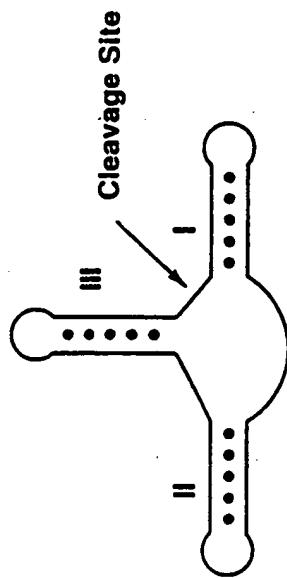
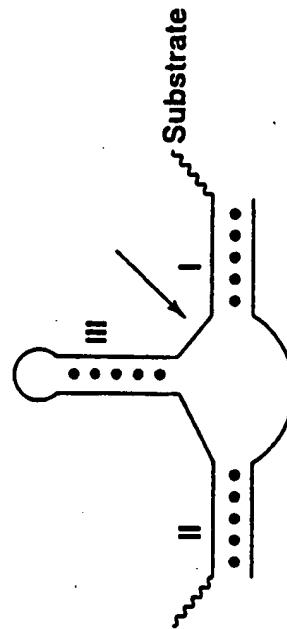


FIG. 2a.



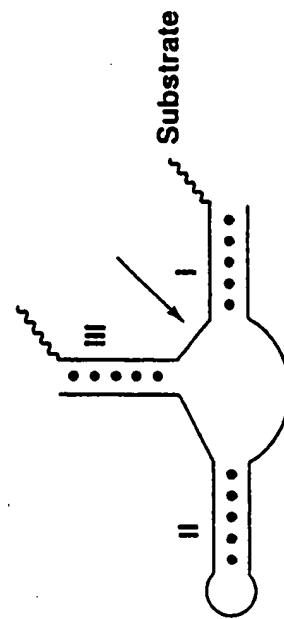
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FIG. 2b.



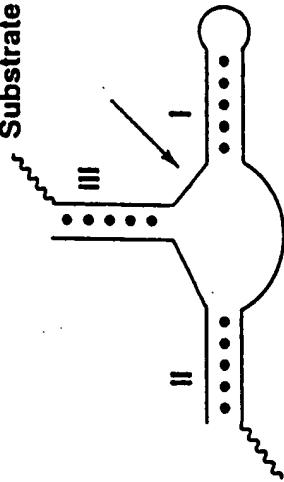
b

FIG. 2c.



c

FIG. 2d.



d

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Substrate RNA

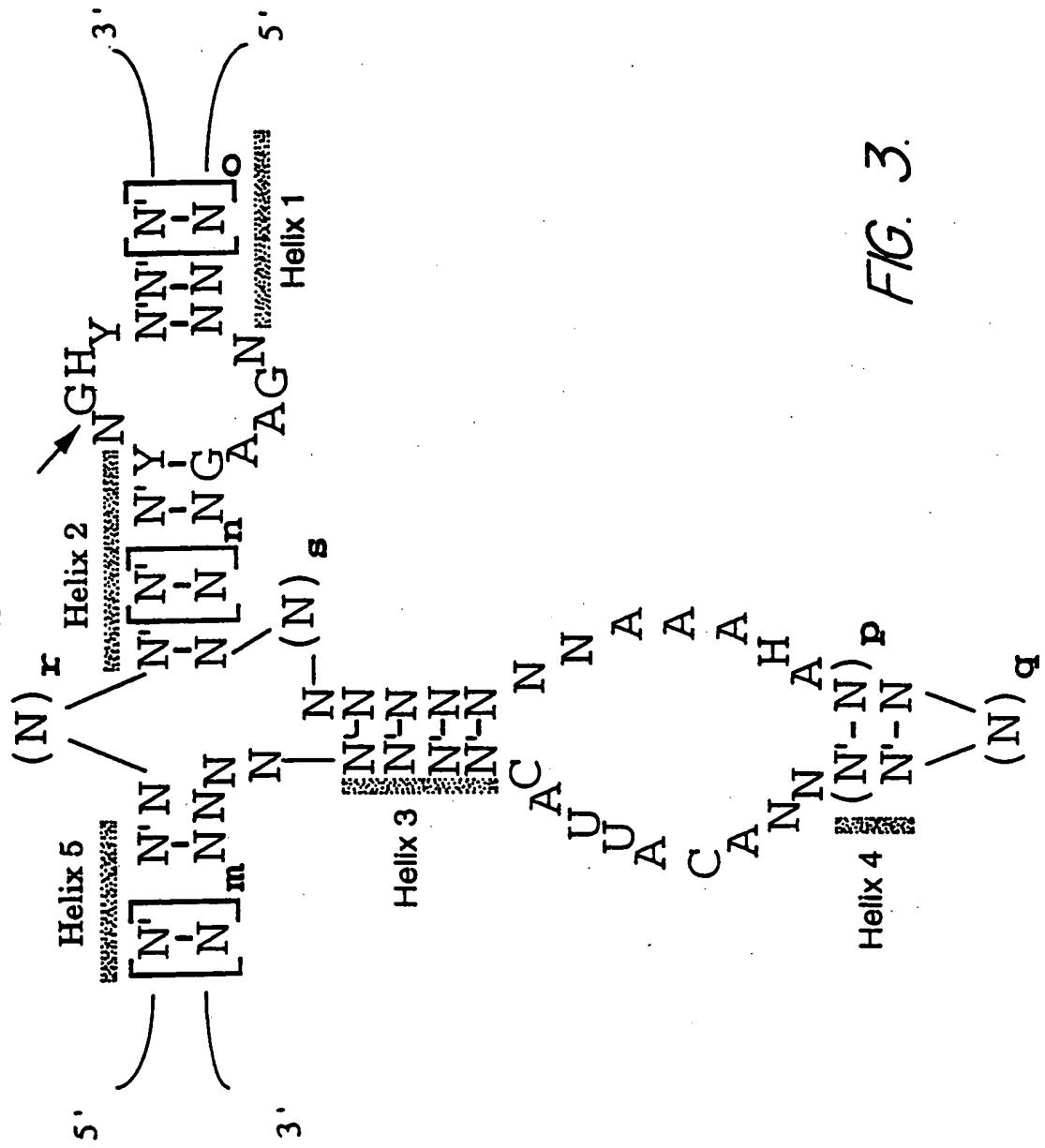
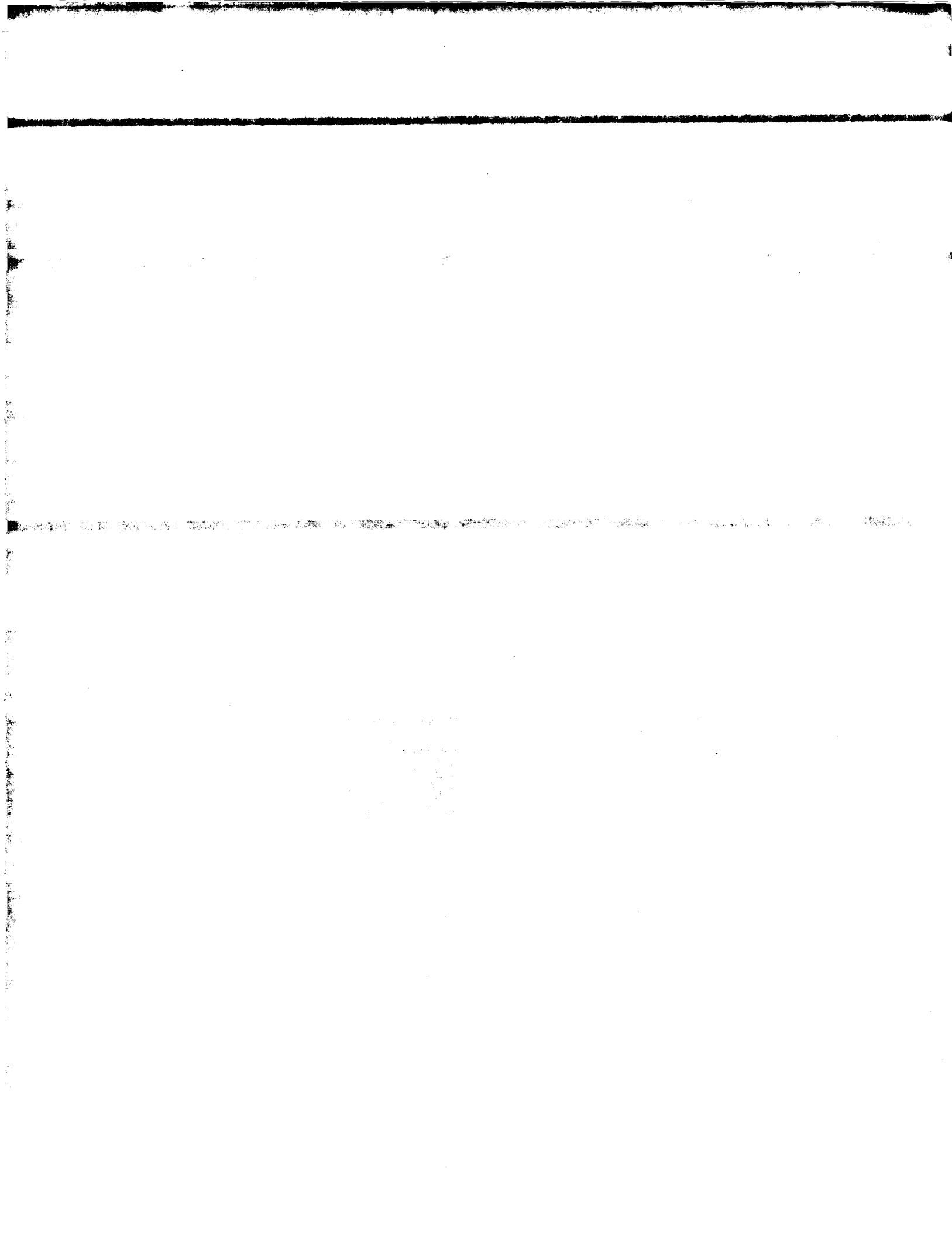


FIG. 3.



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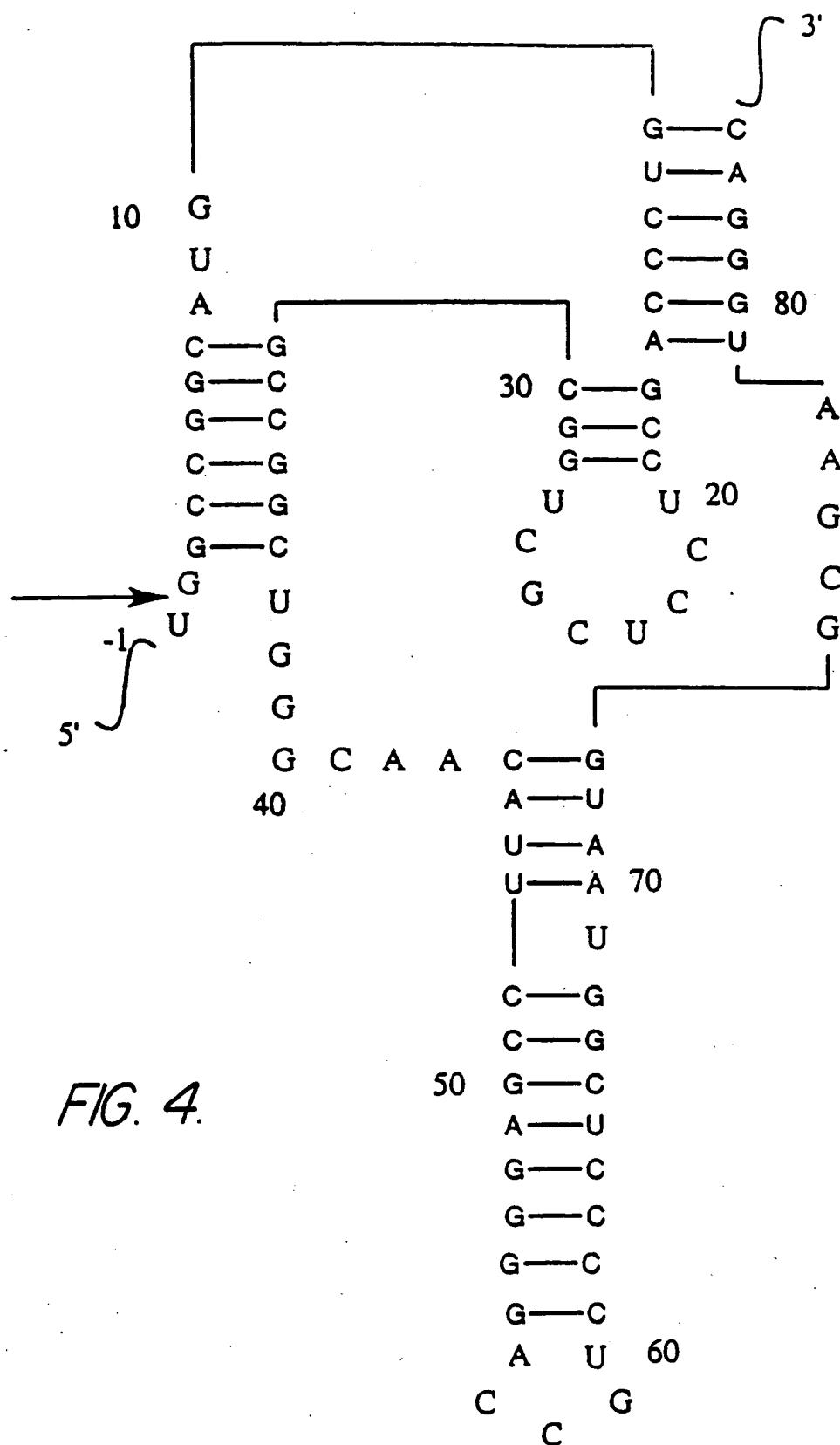
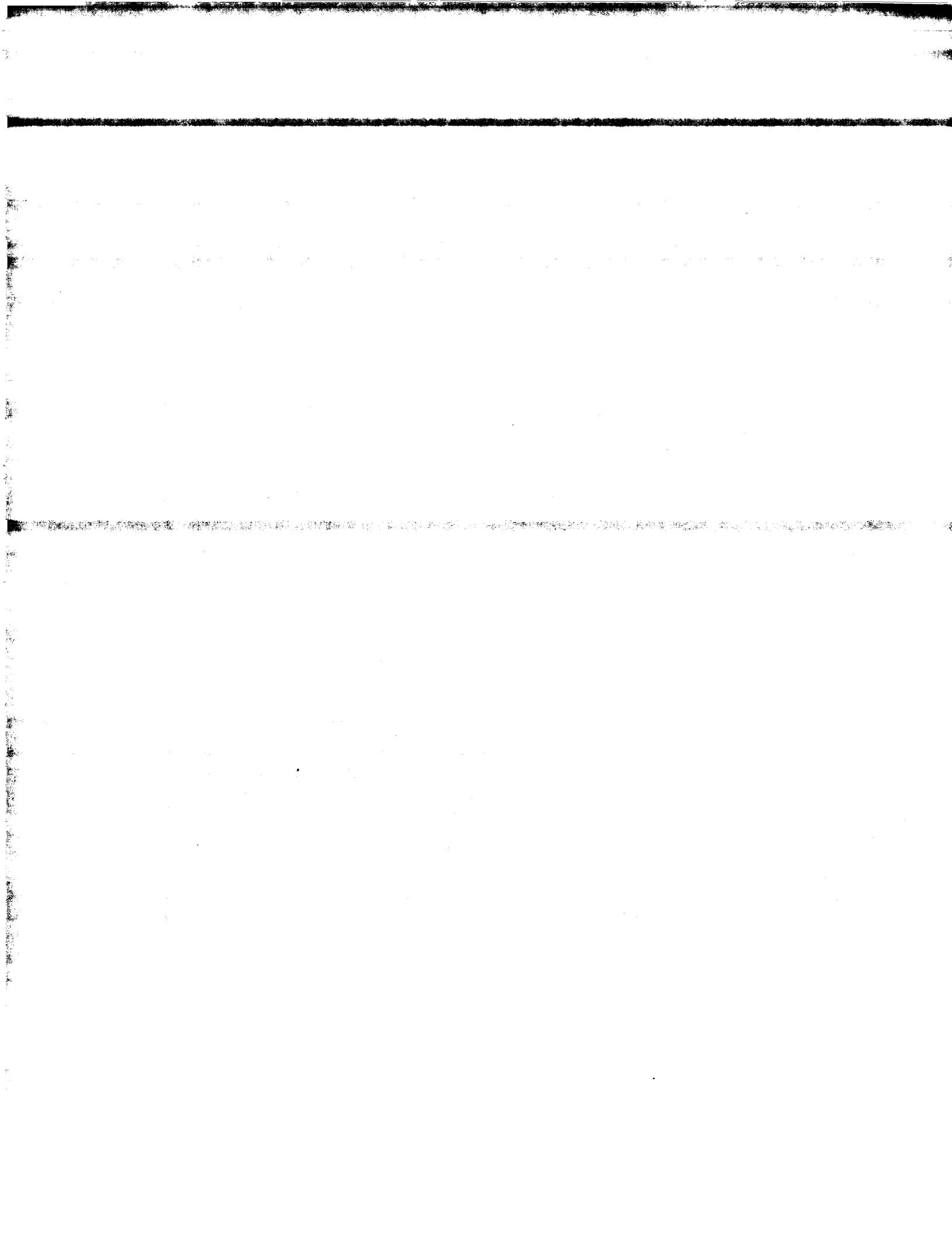
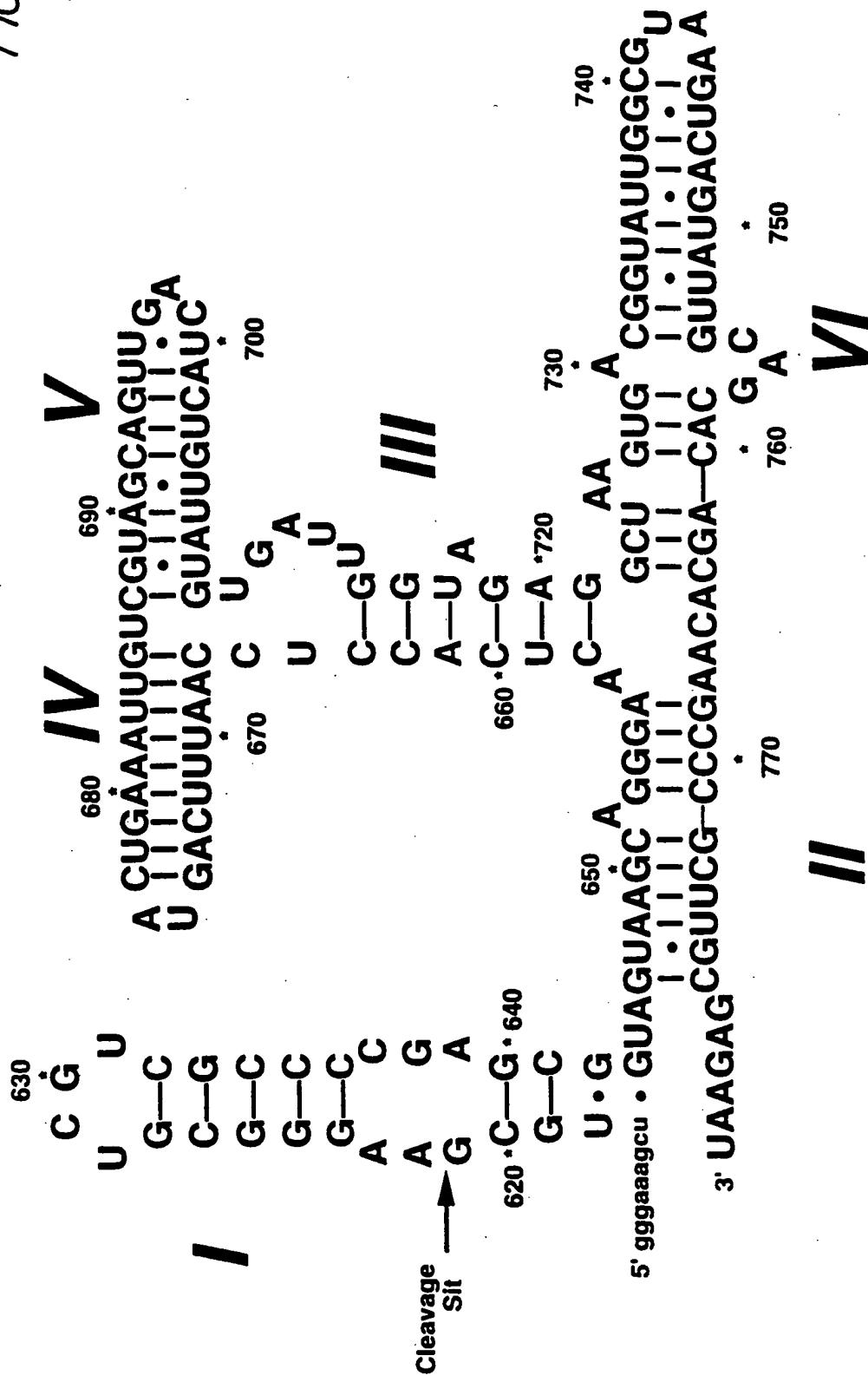


FIG. 4.



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FIG. 5.



SUBSTITUTE SHEET (RULE 26)

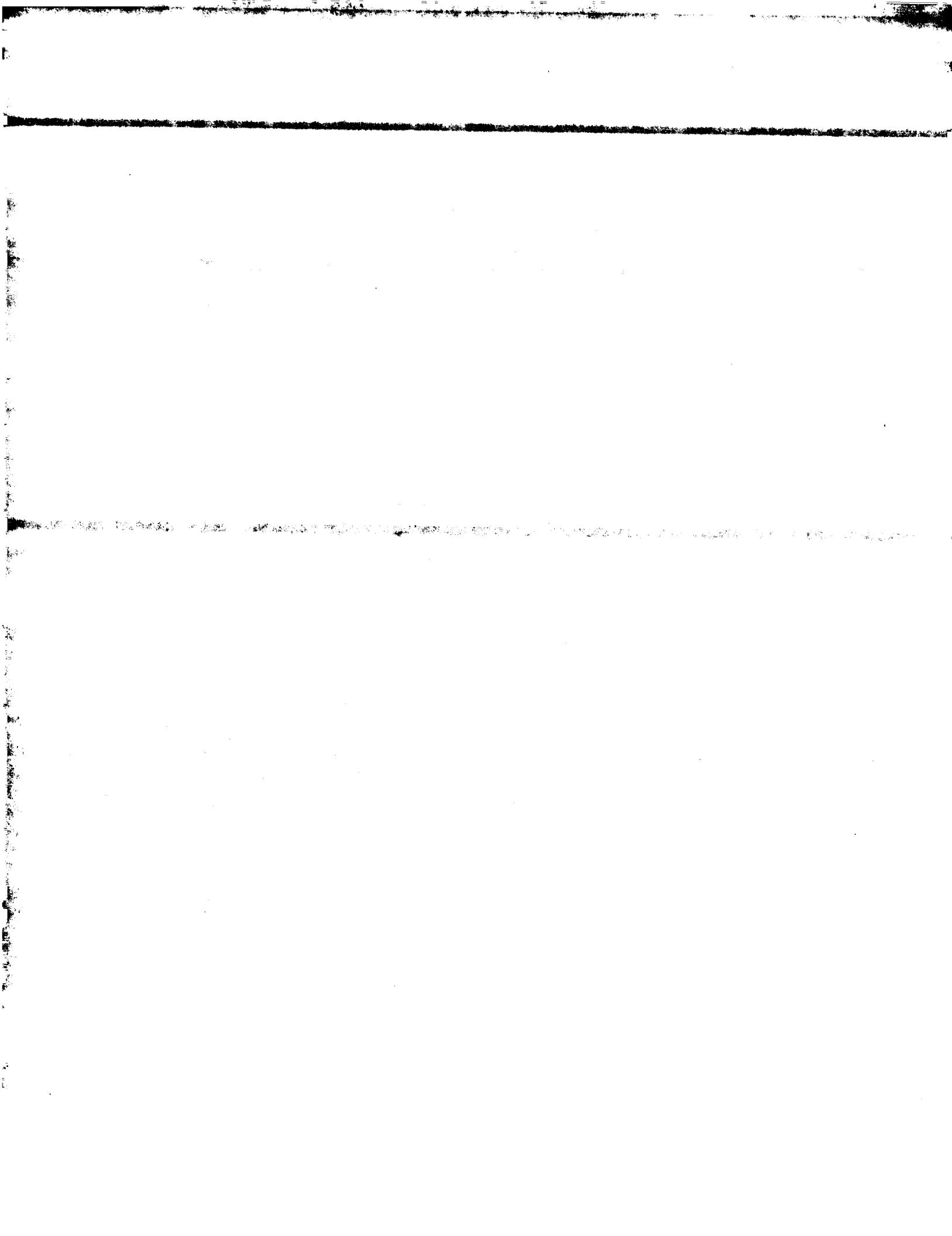
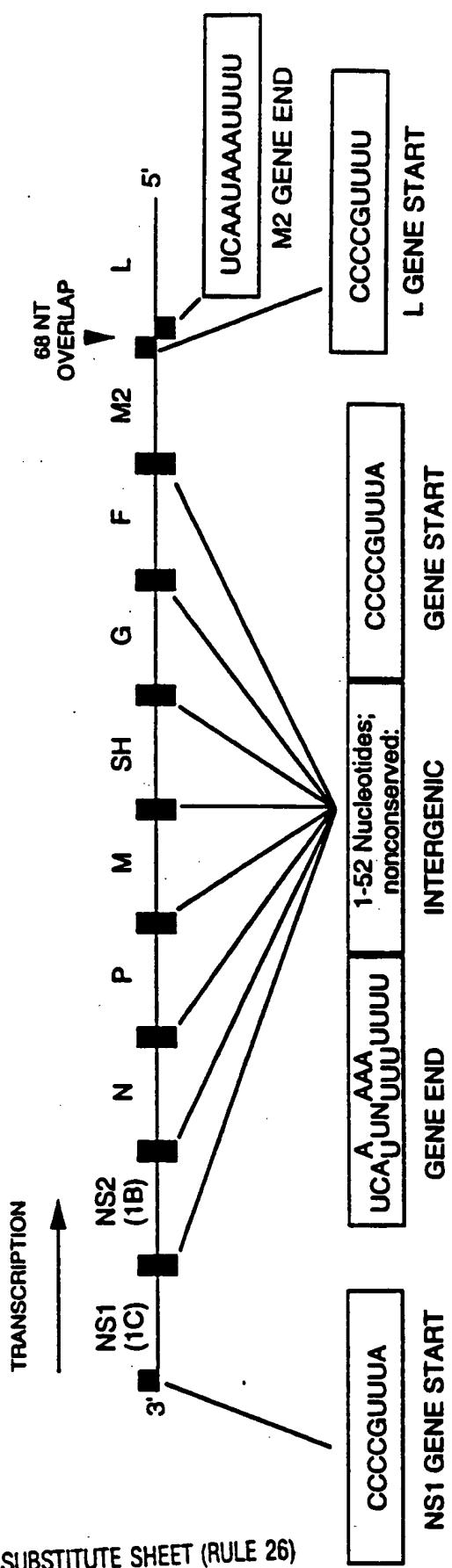
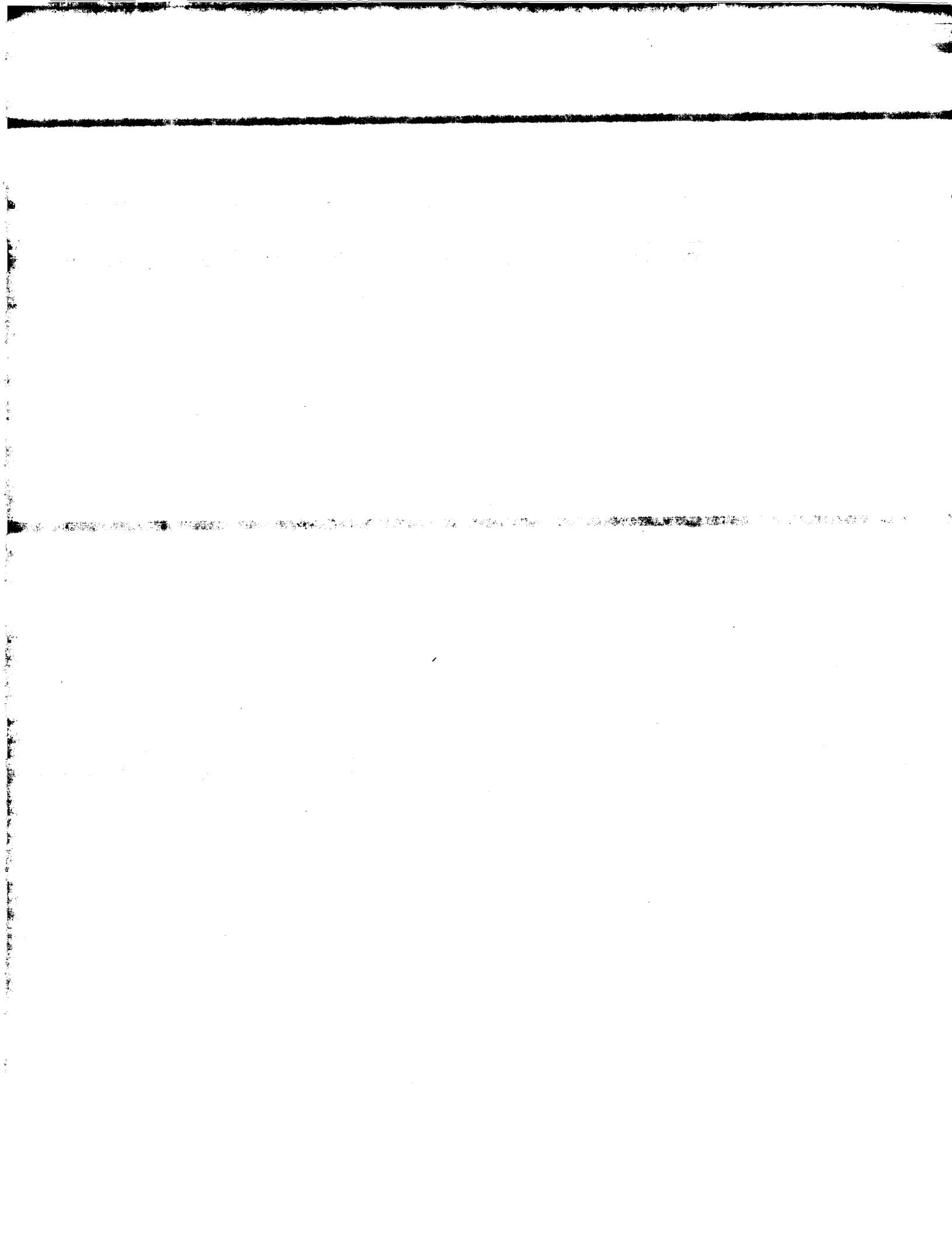


FIG. 6.

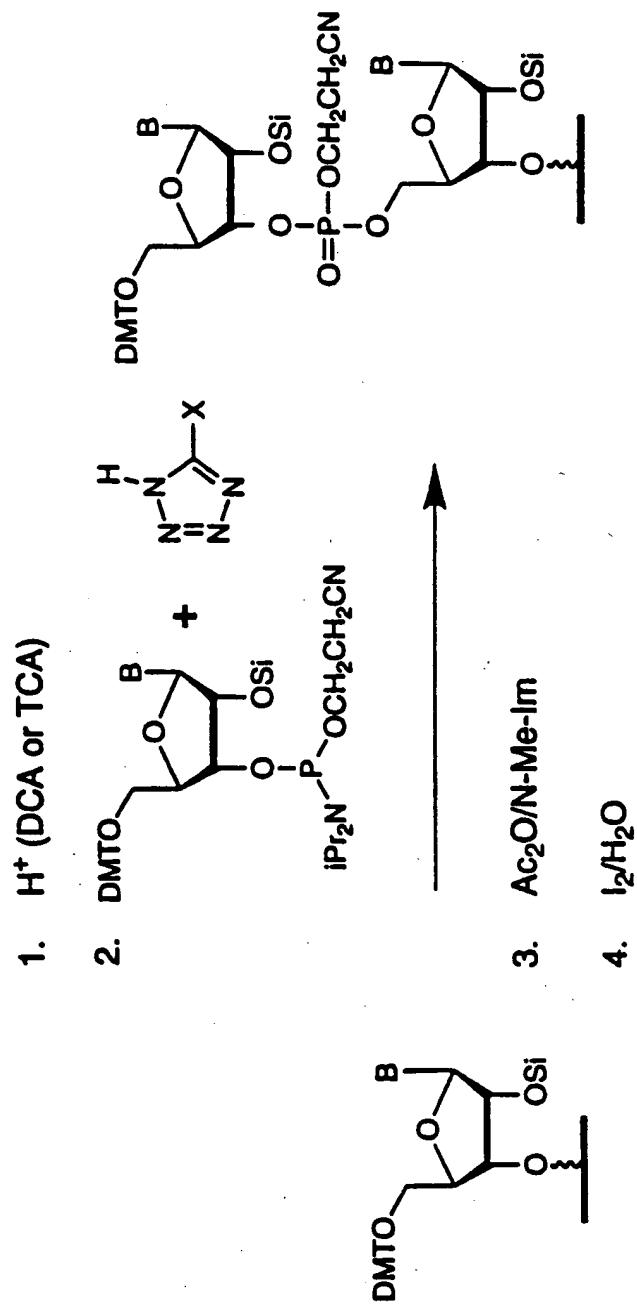


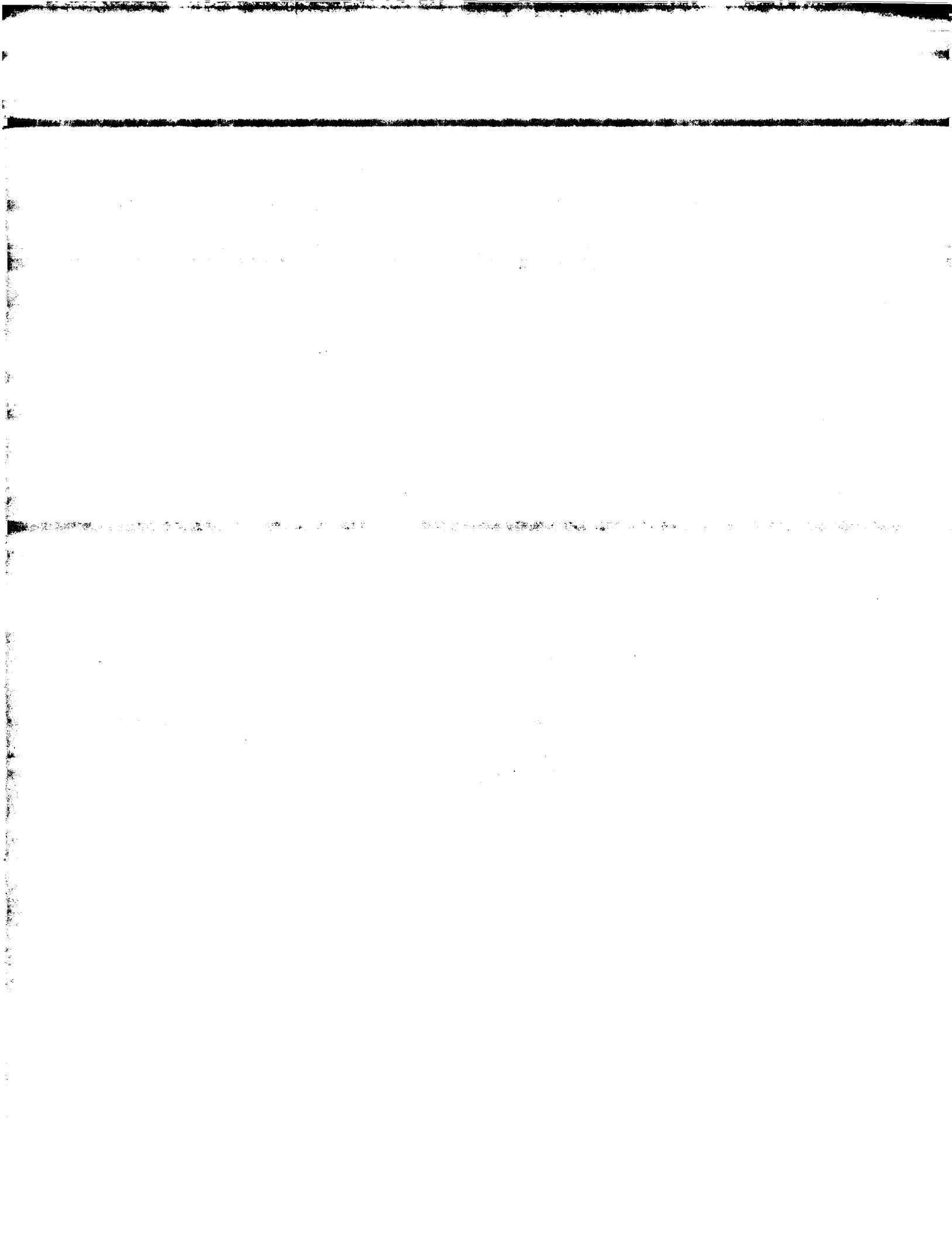
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FIG. 7.





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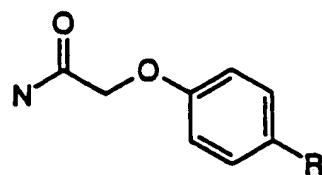
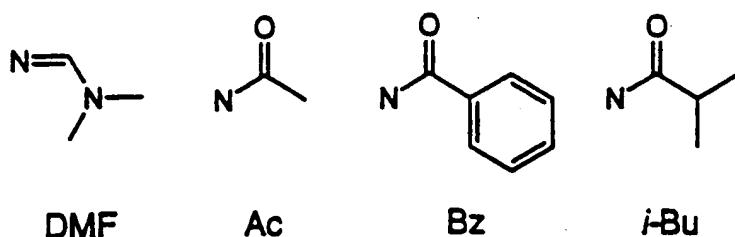
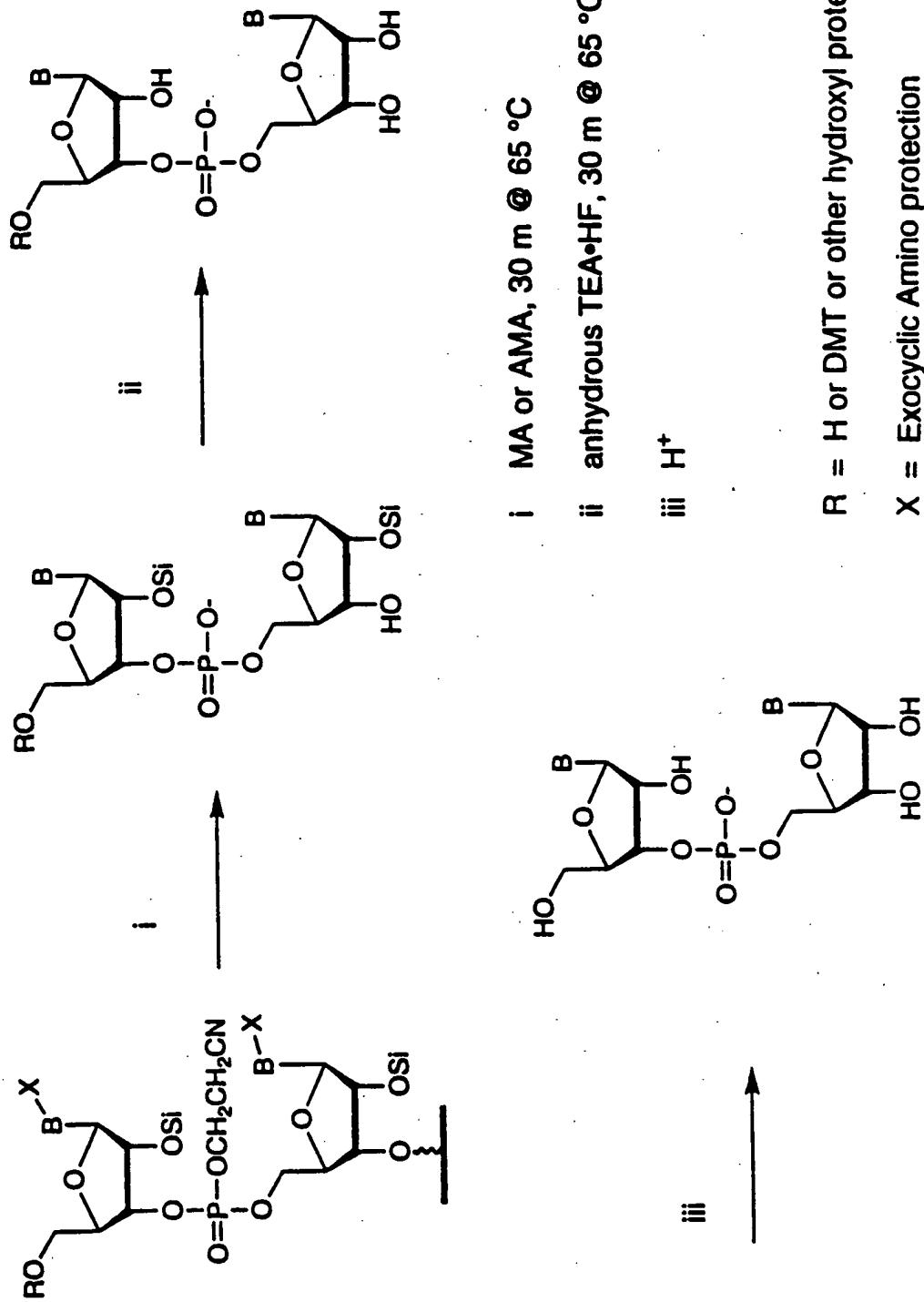


FIG. 8.

 $R = H = PAC$ $R = tBu = TAC$ $R = iPr = iPPAC$

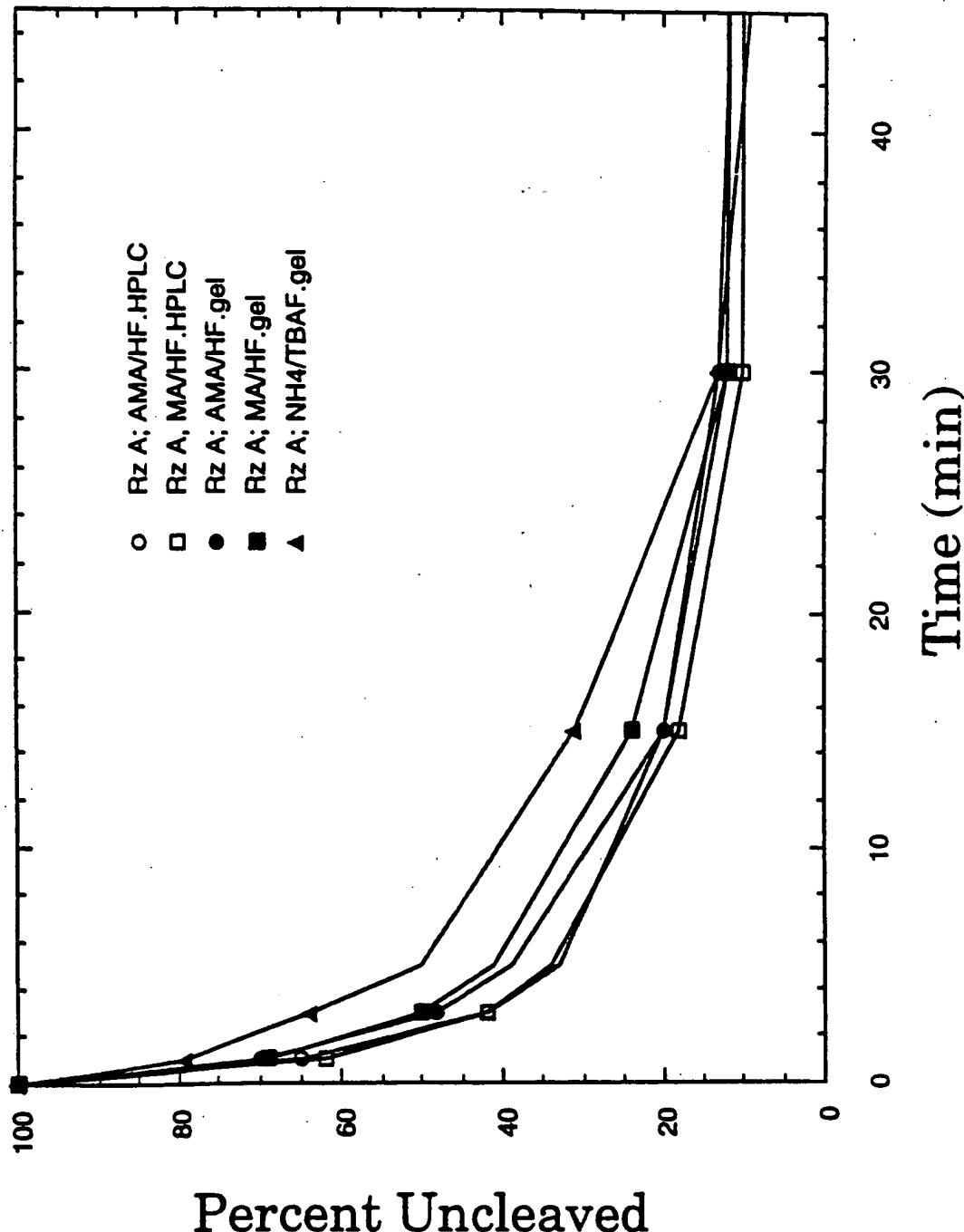
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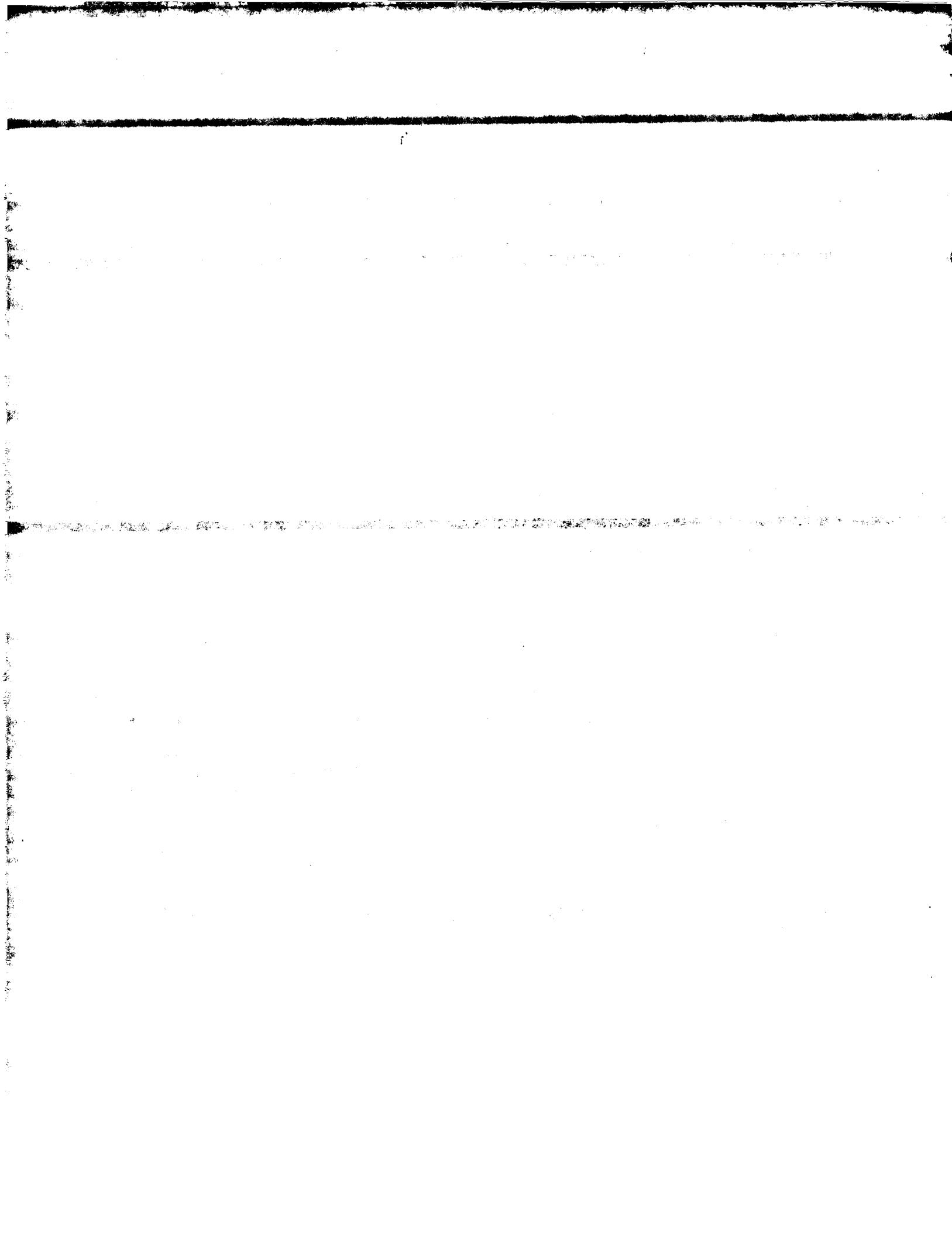
FIG. 9.



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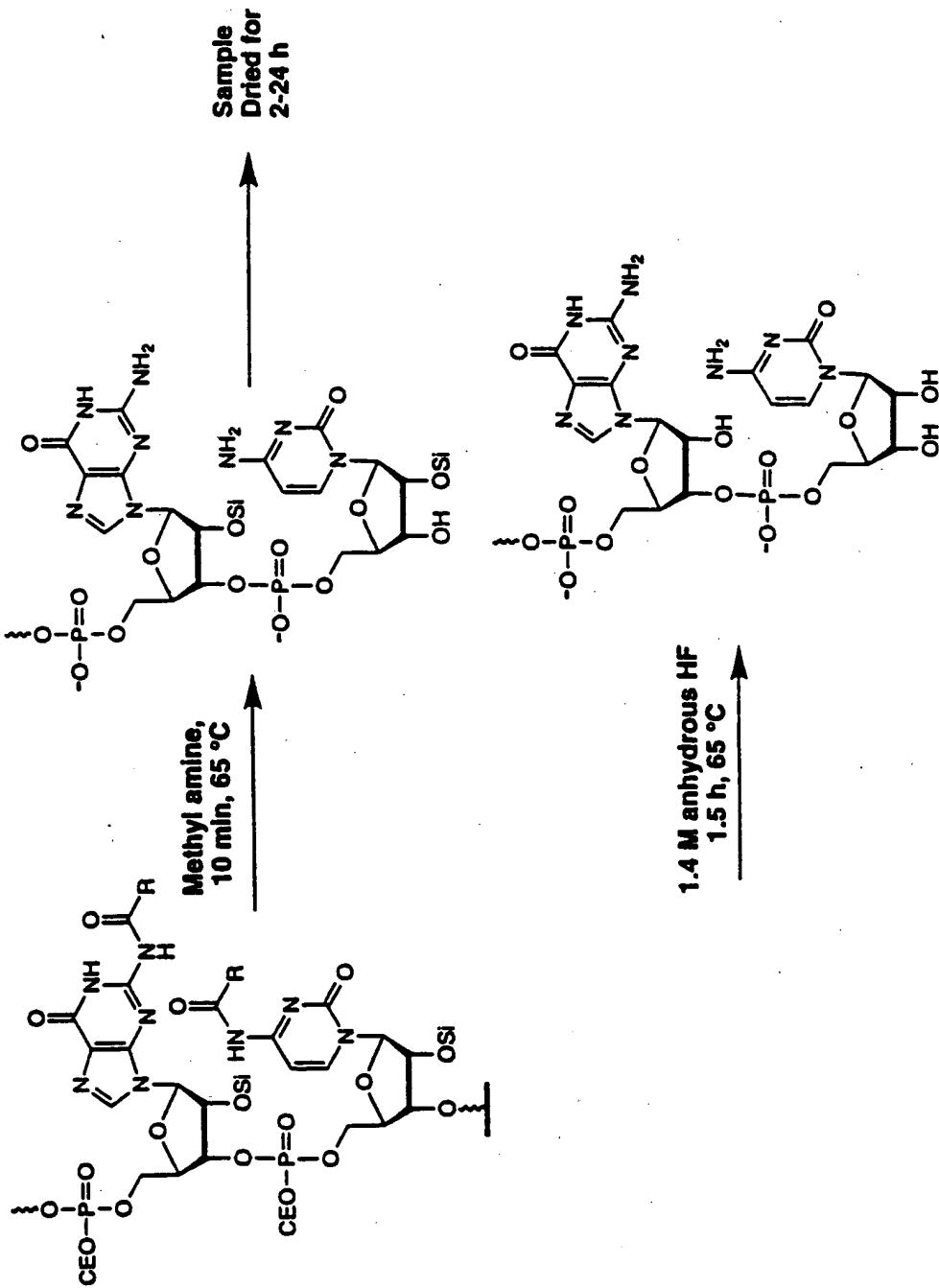
FIG. 10.

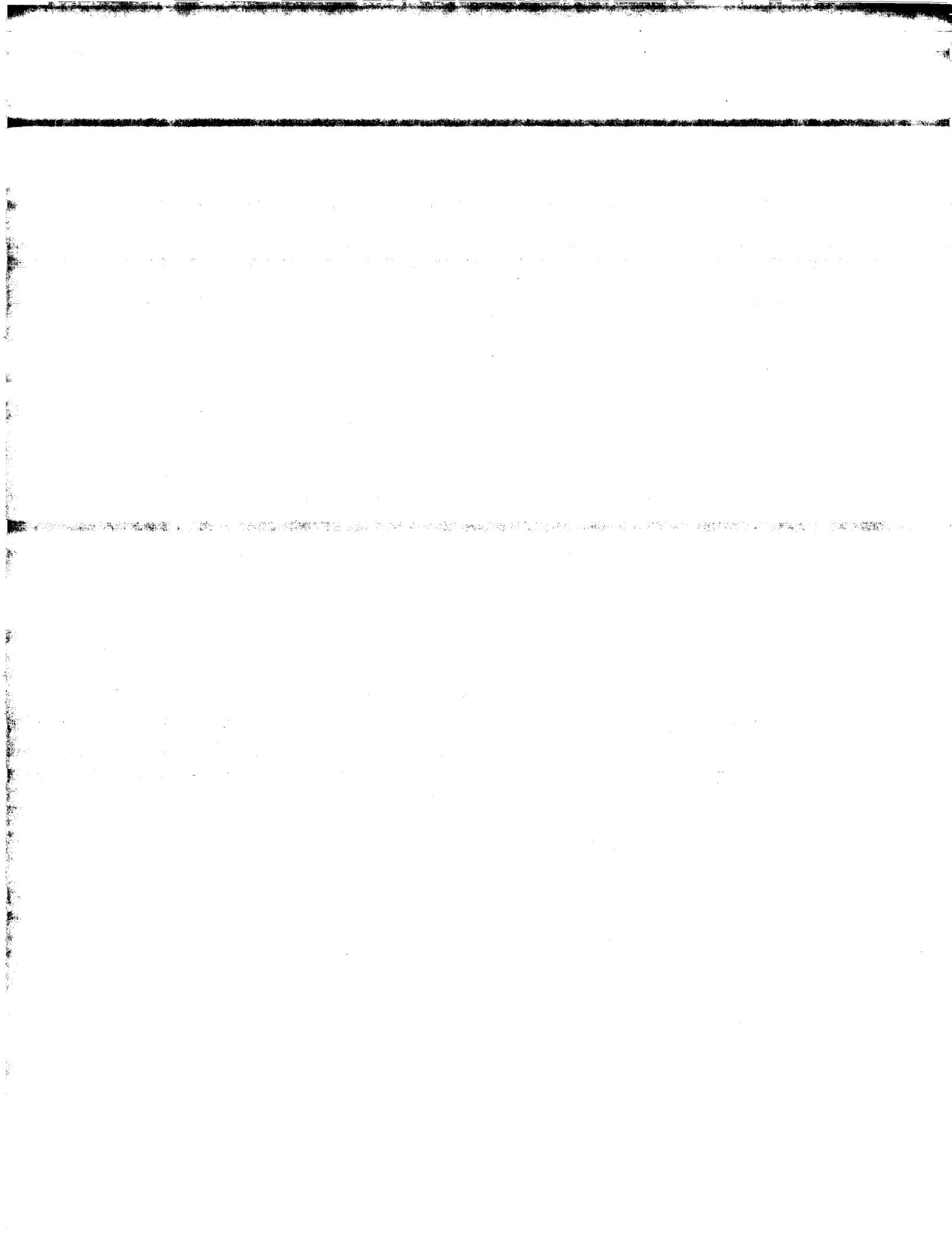




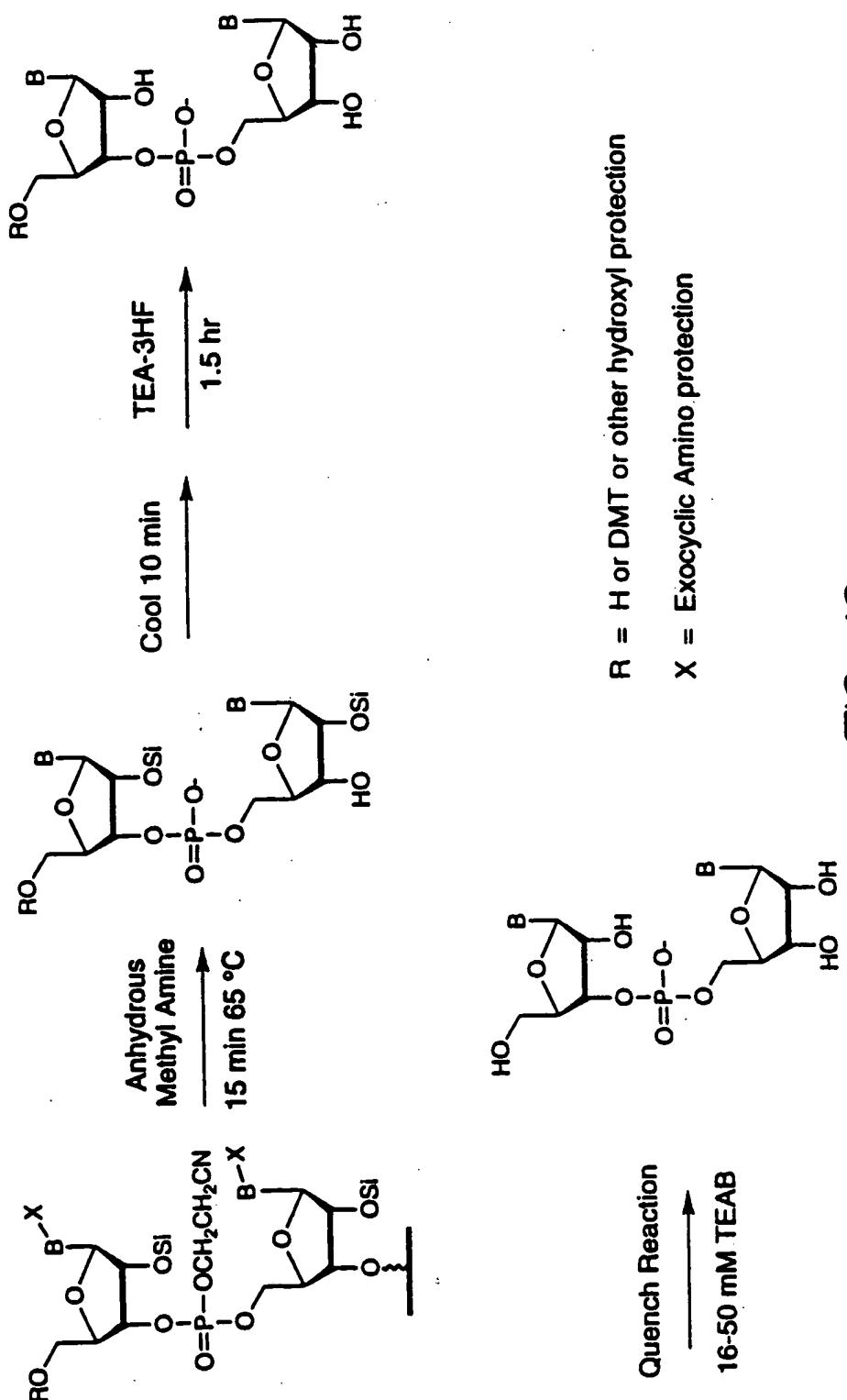
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FIG. 11.

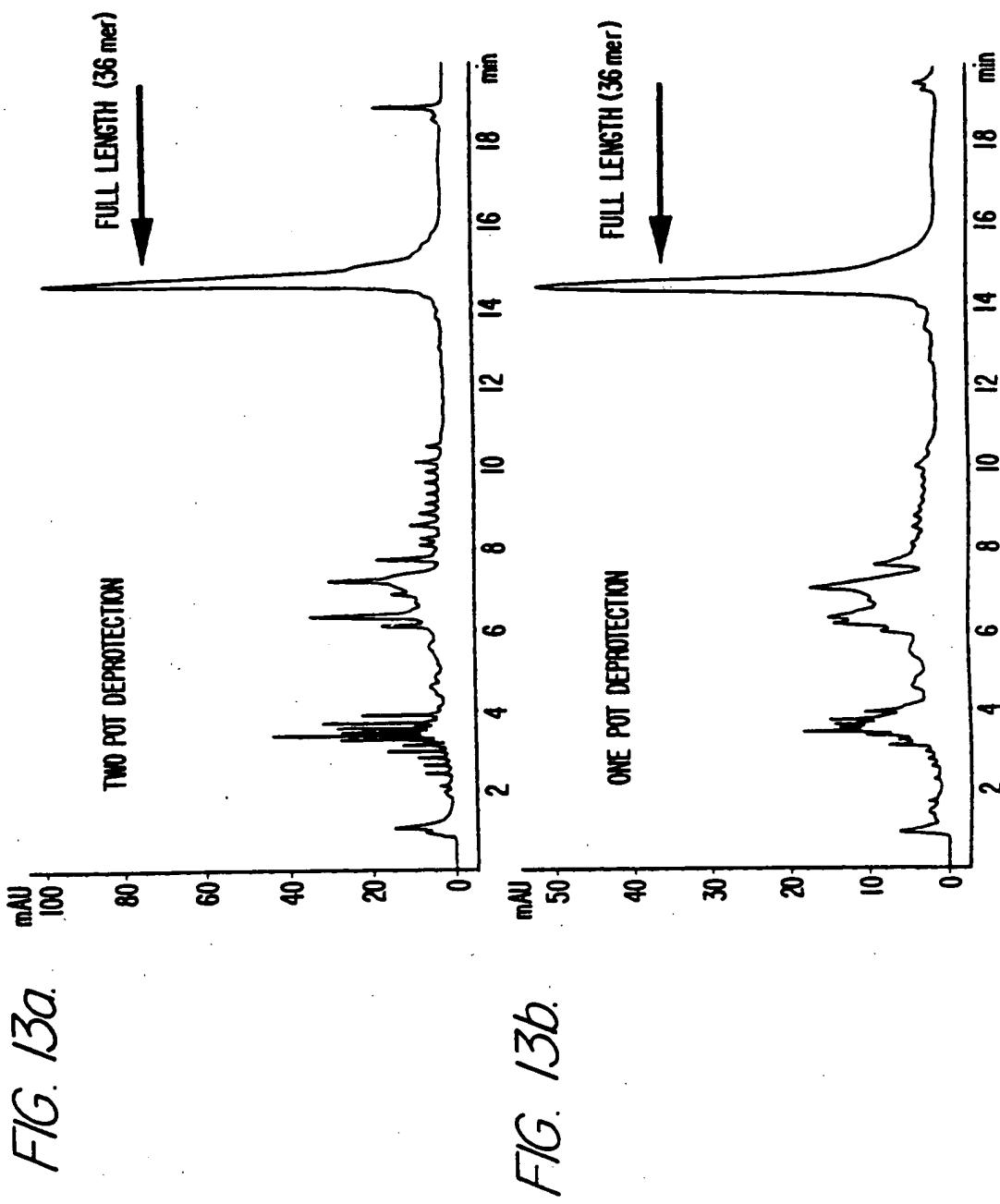


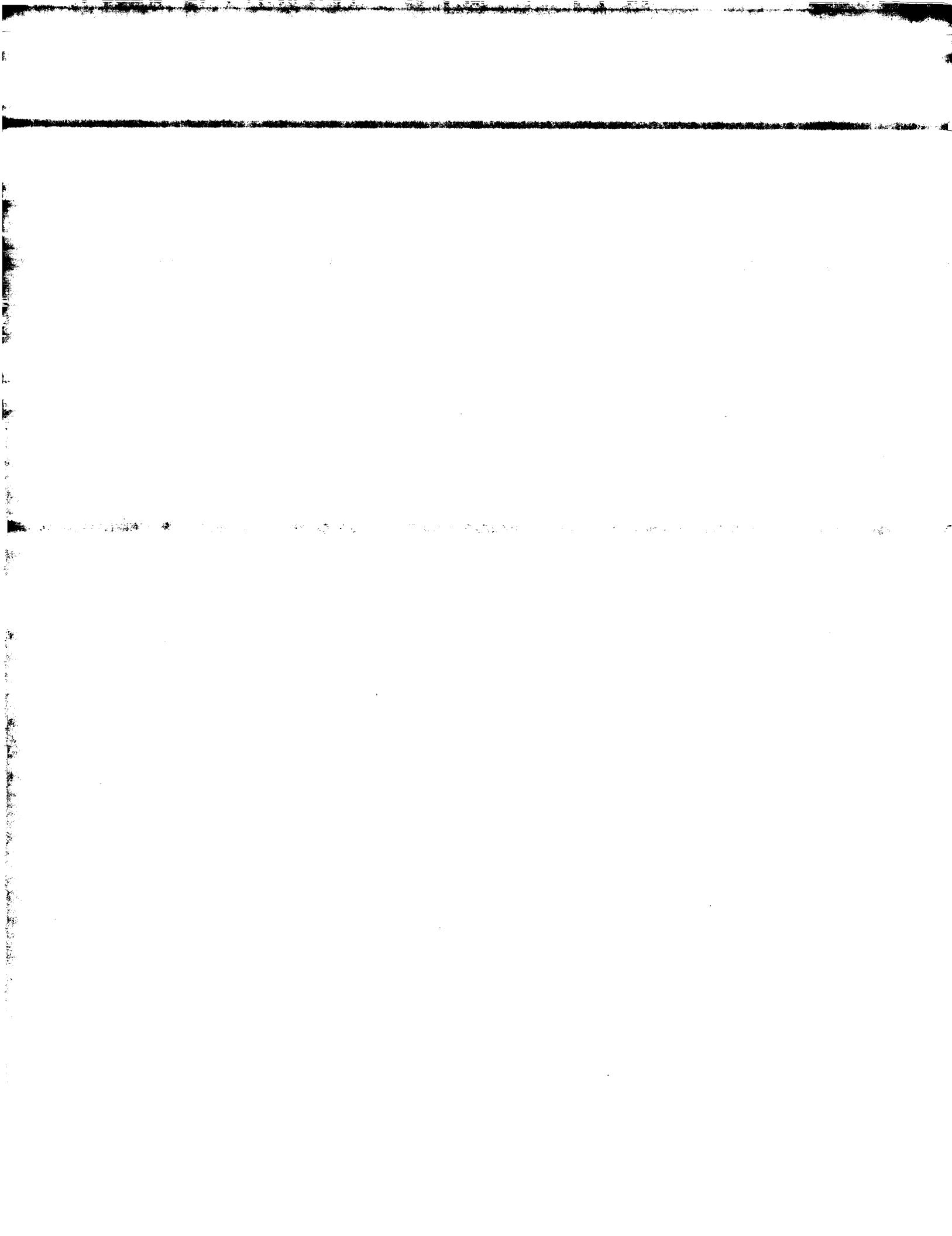


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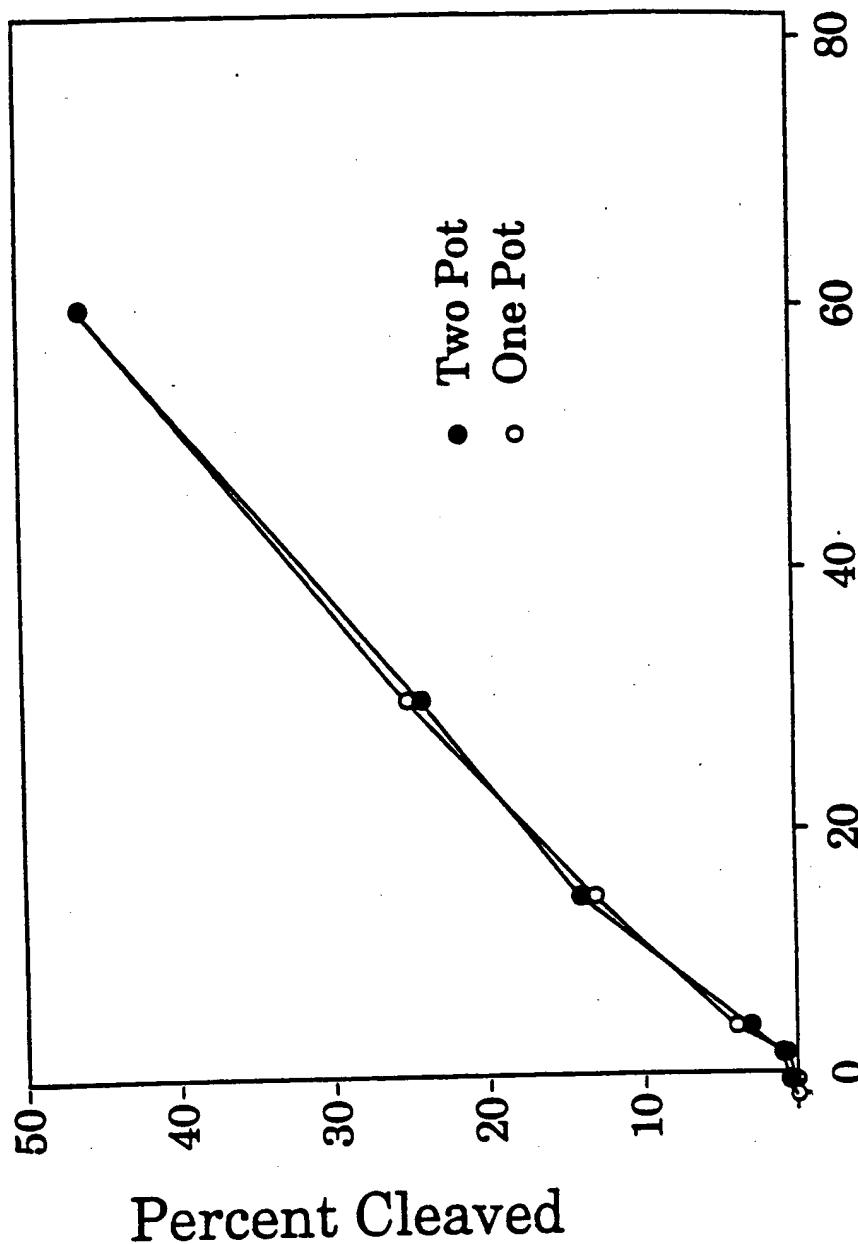
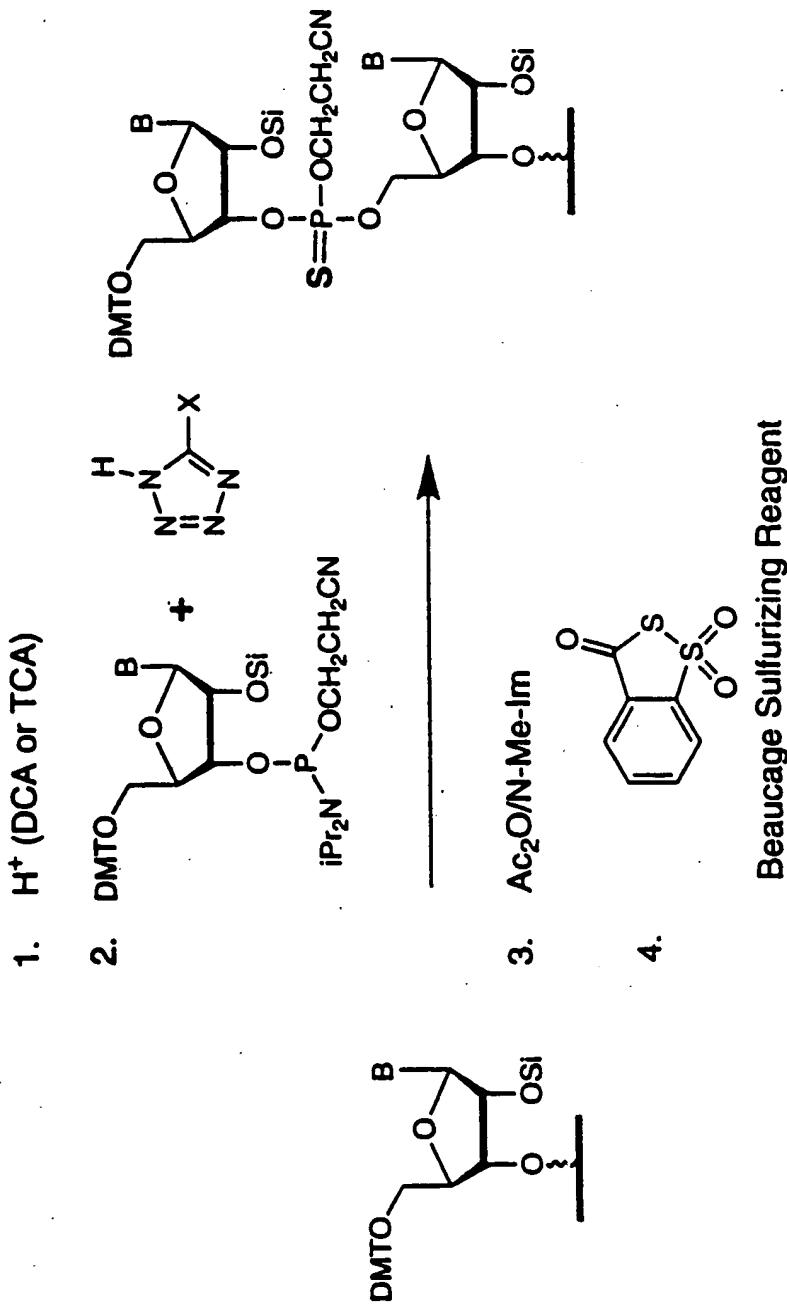


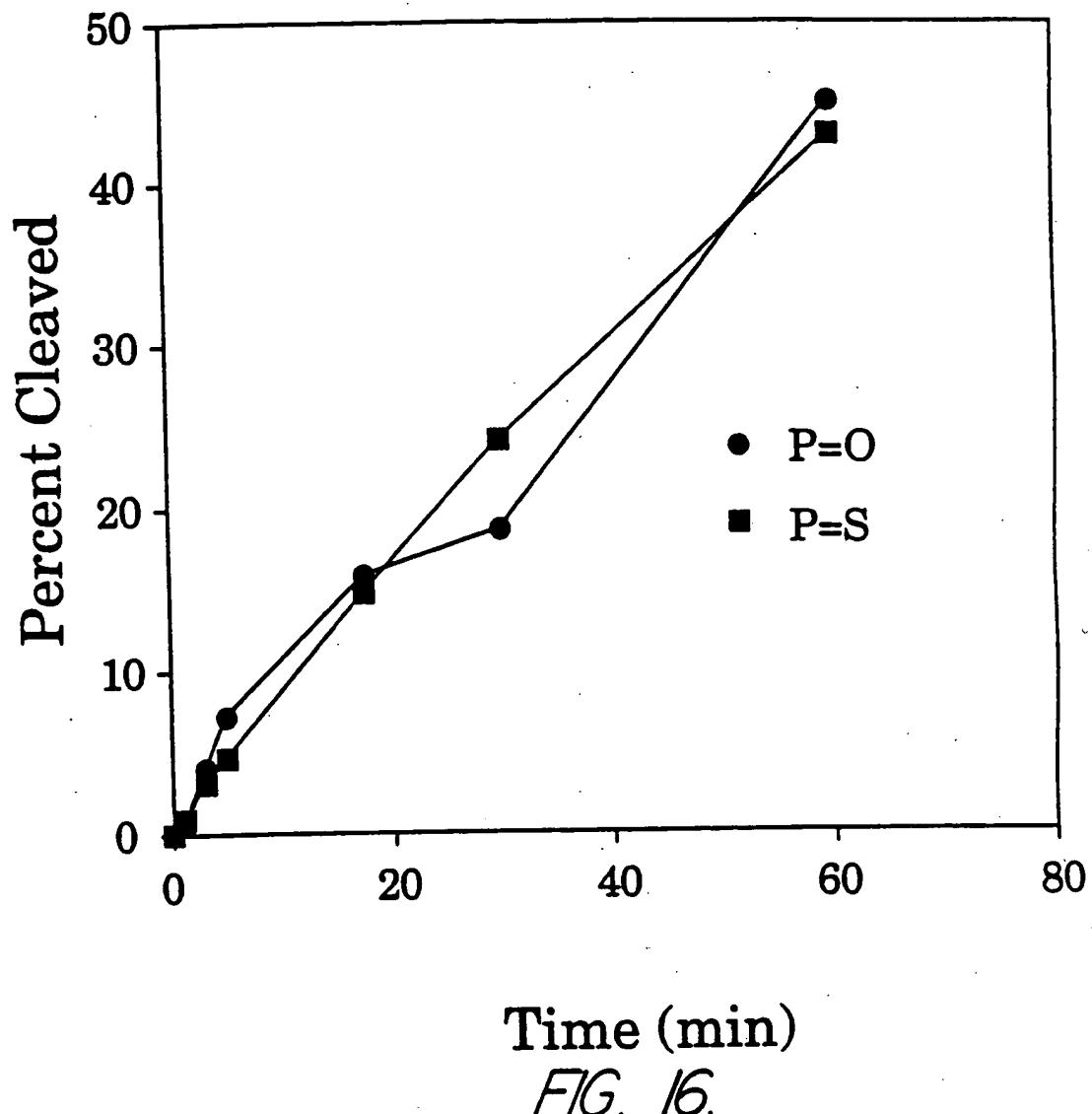
FIG. 14.

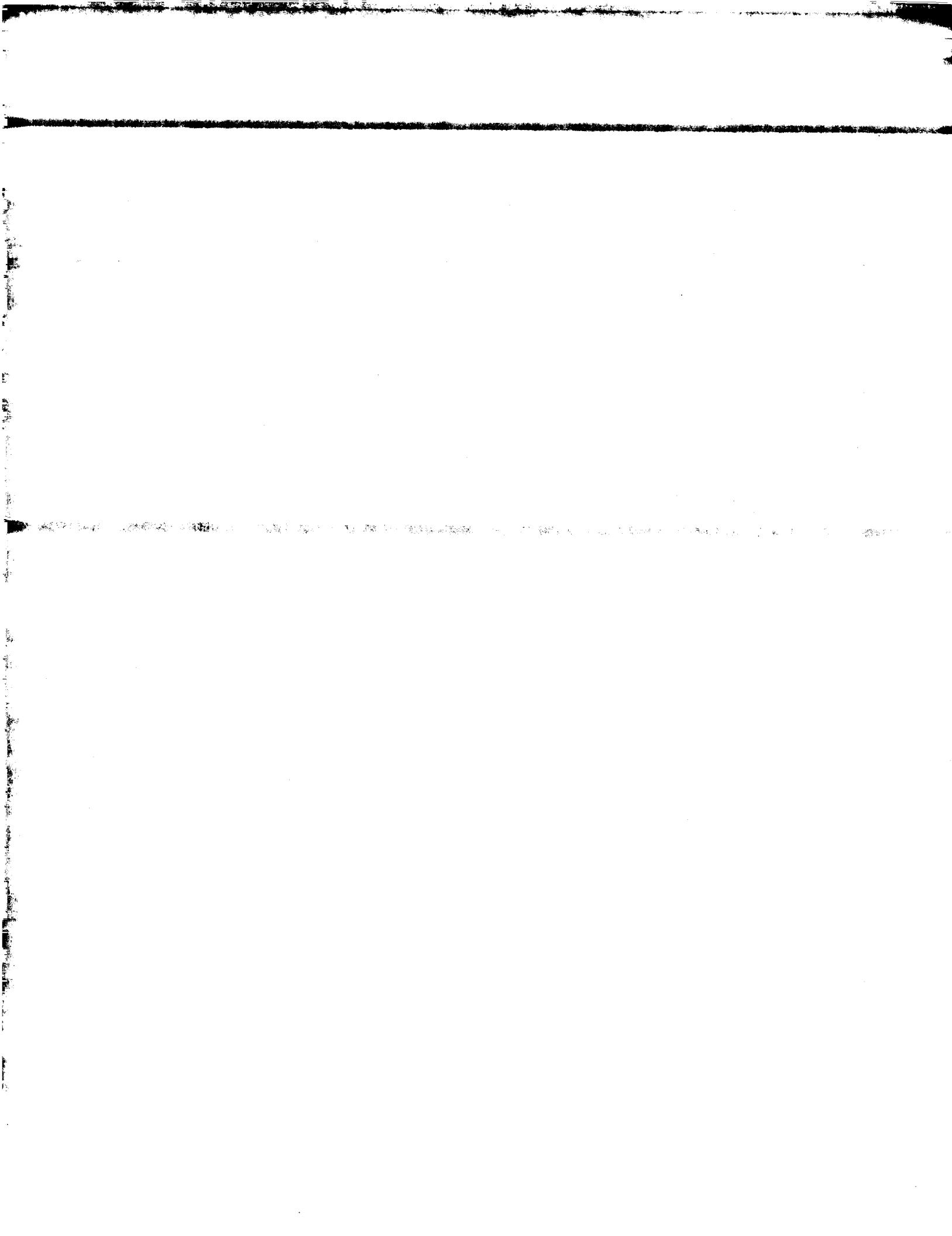
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FIG. 15.

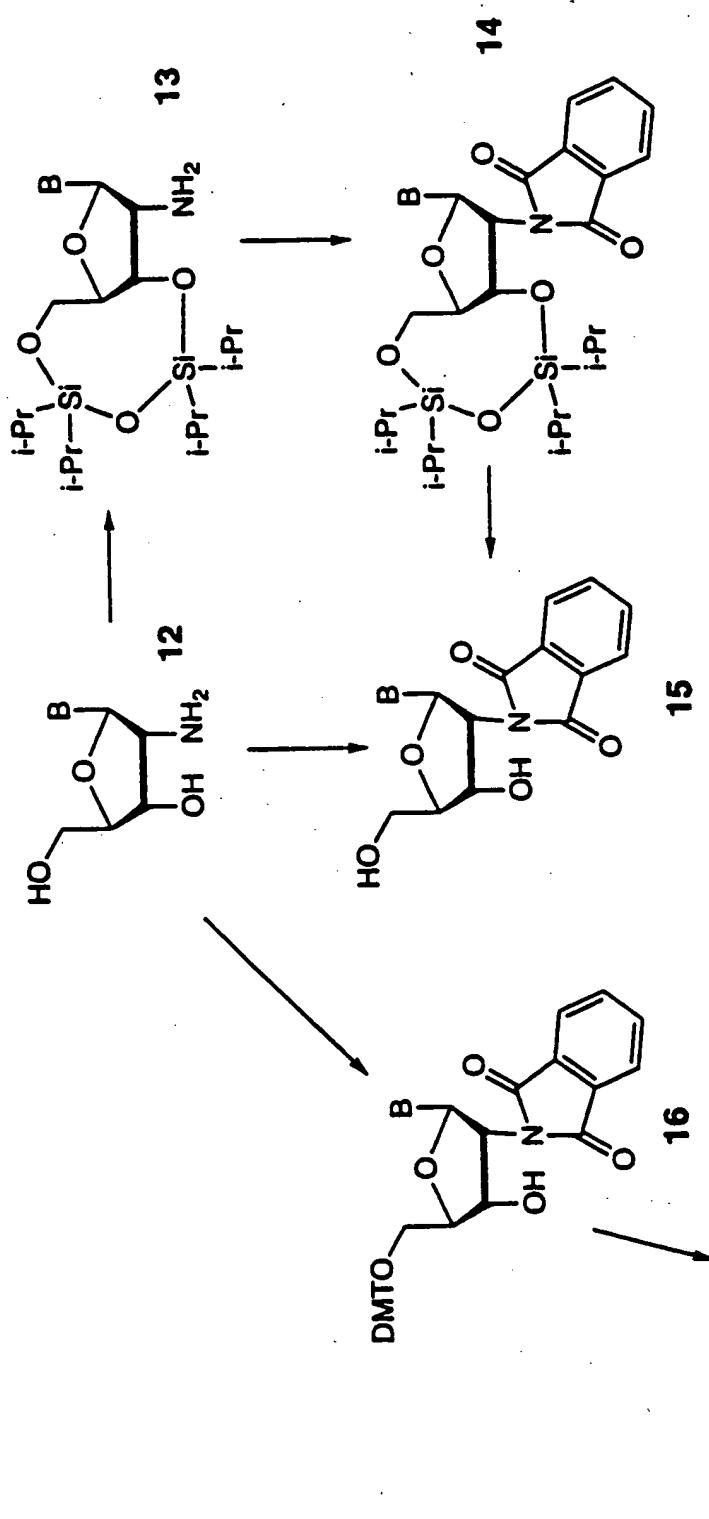


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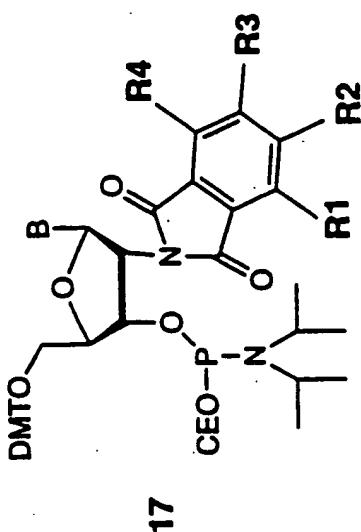


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B = any regular or modified base or abasic
 R1-R4 = alkyl or halogen

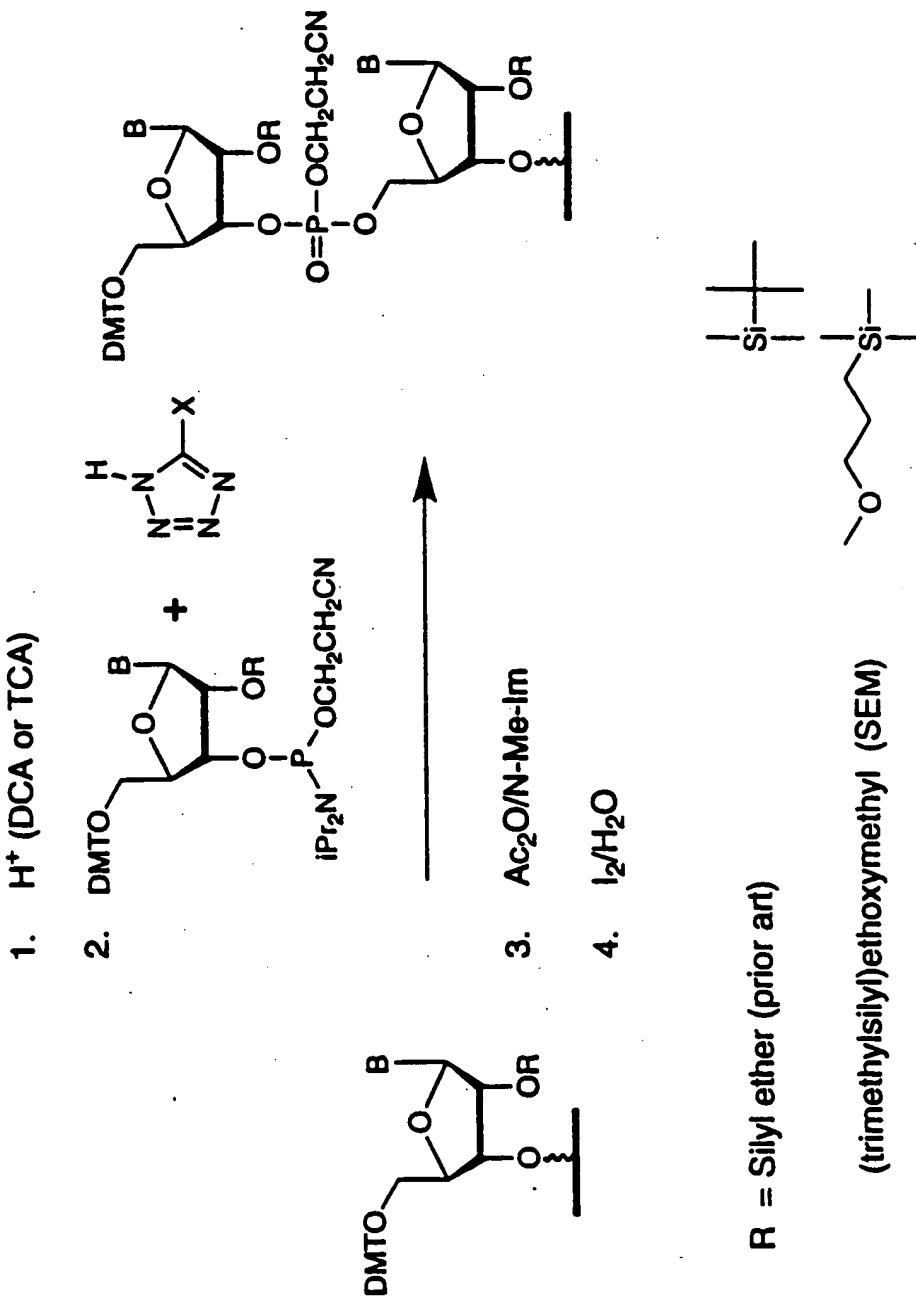
FIG. 17.

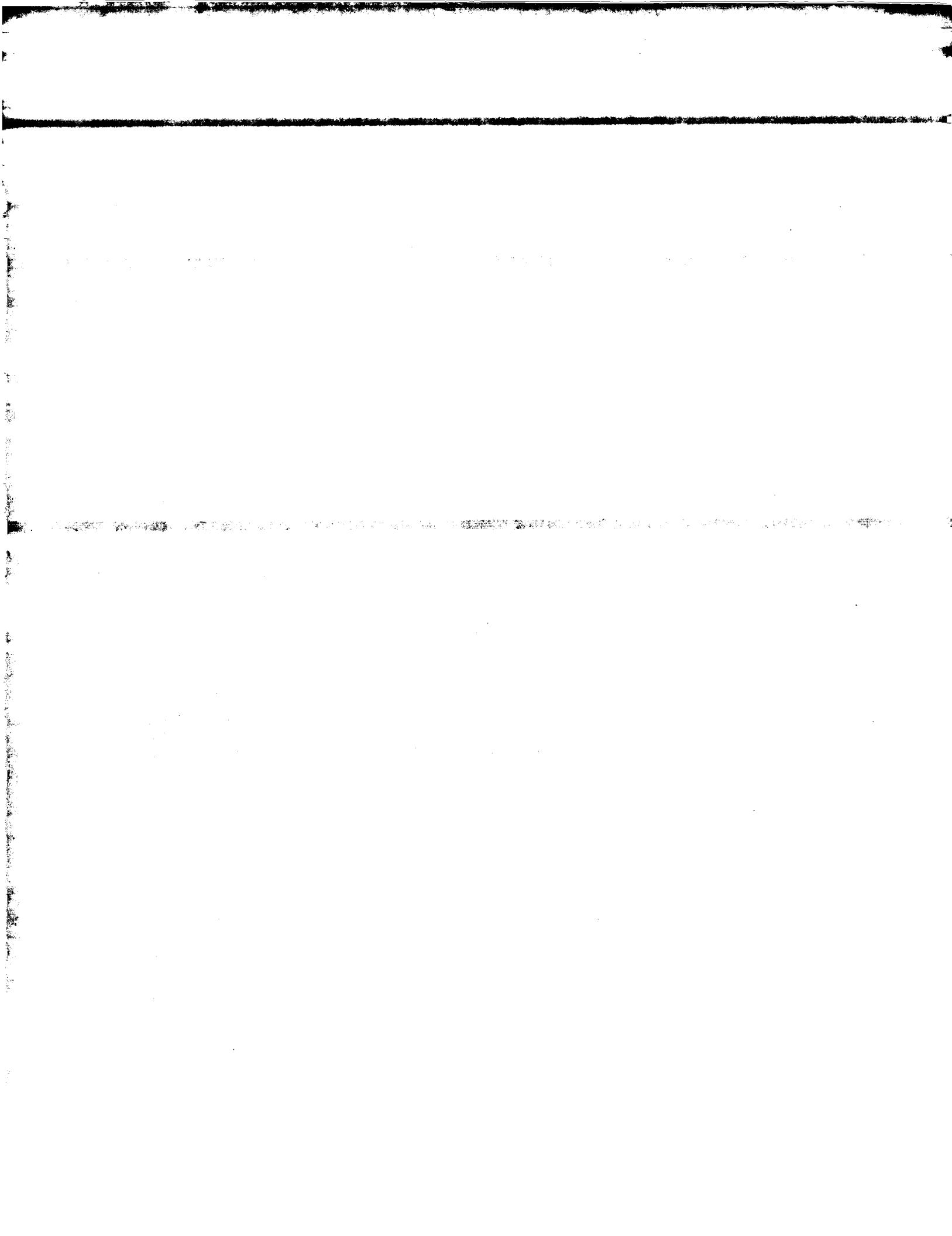




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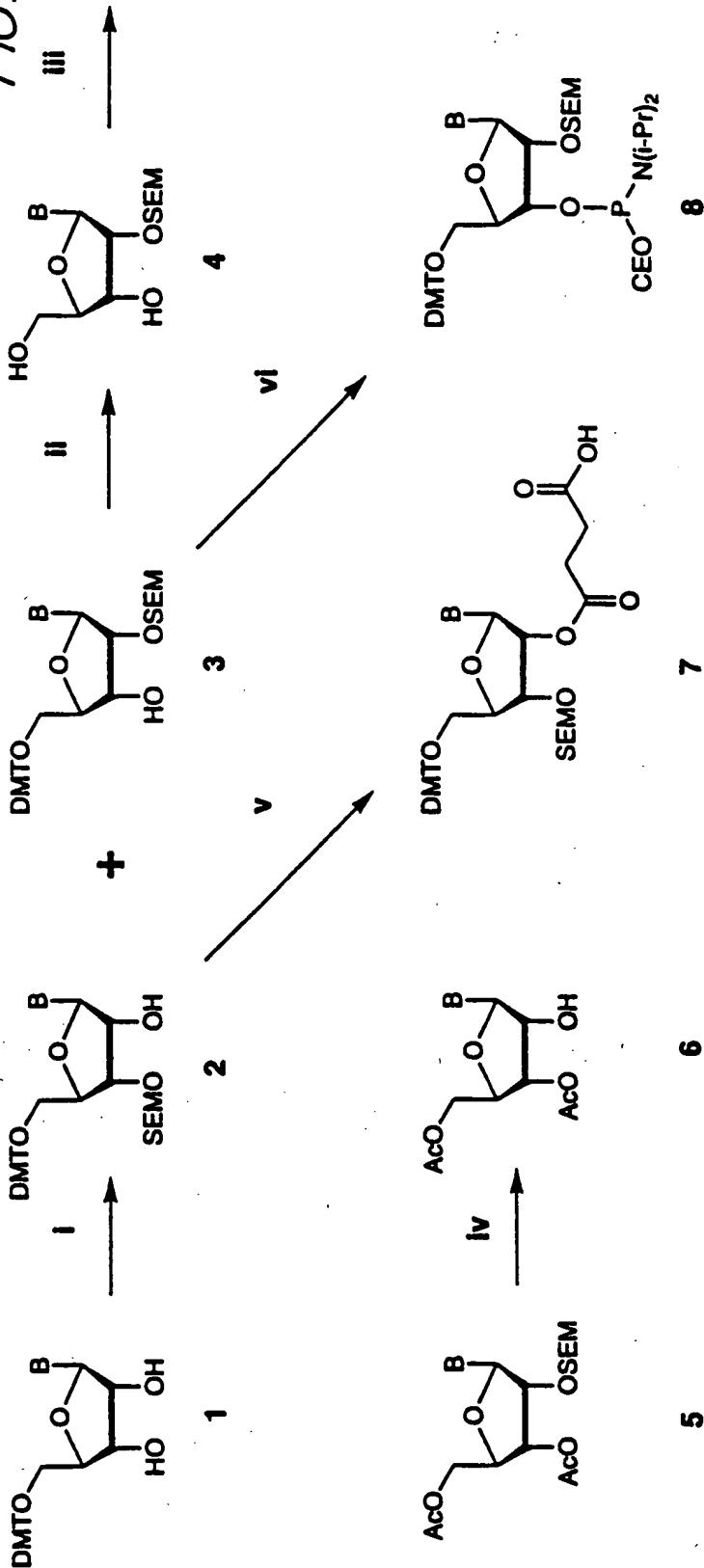
FIG. 18.





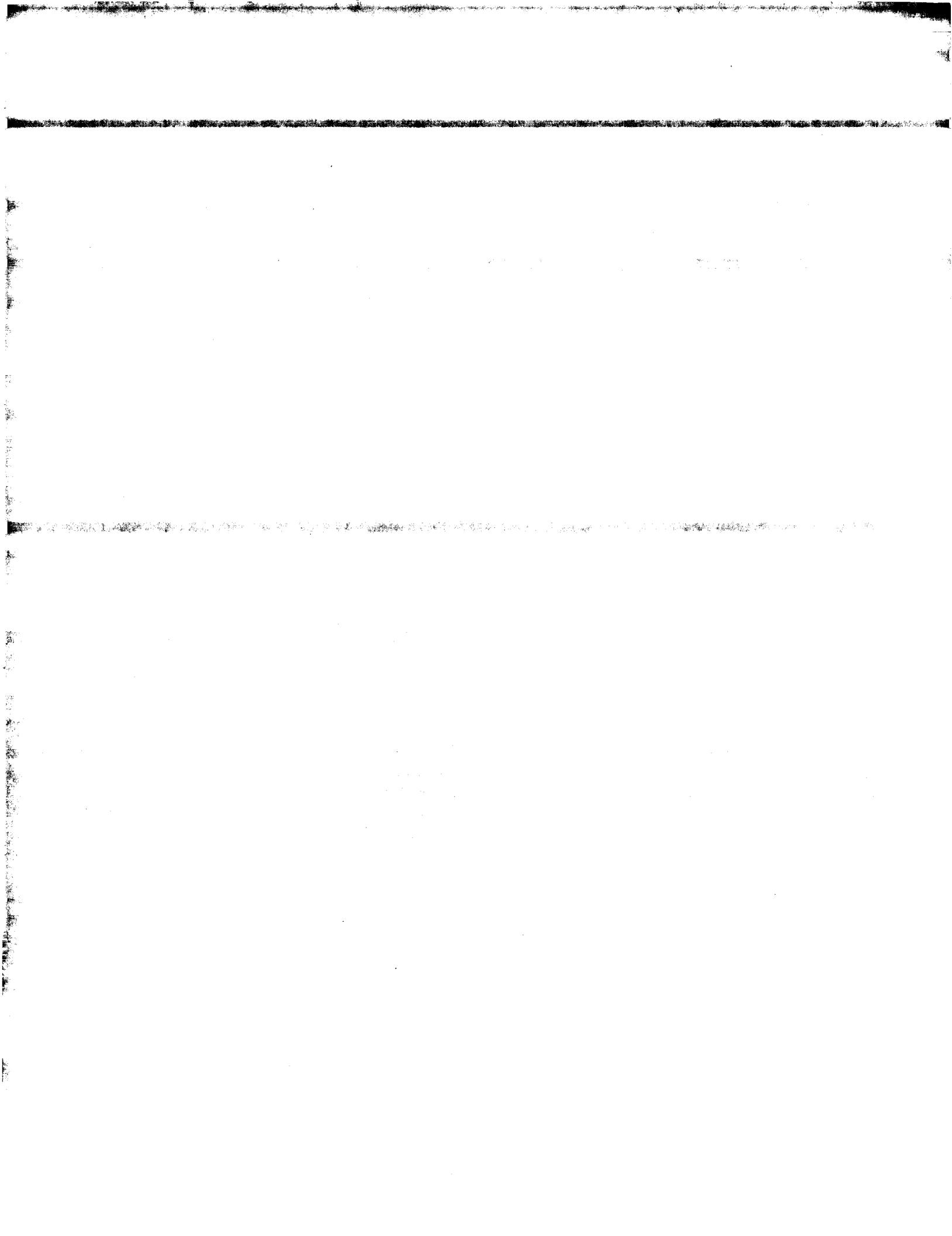
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FIG. 19.

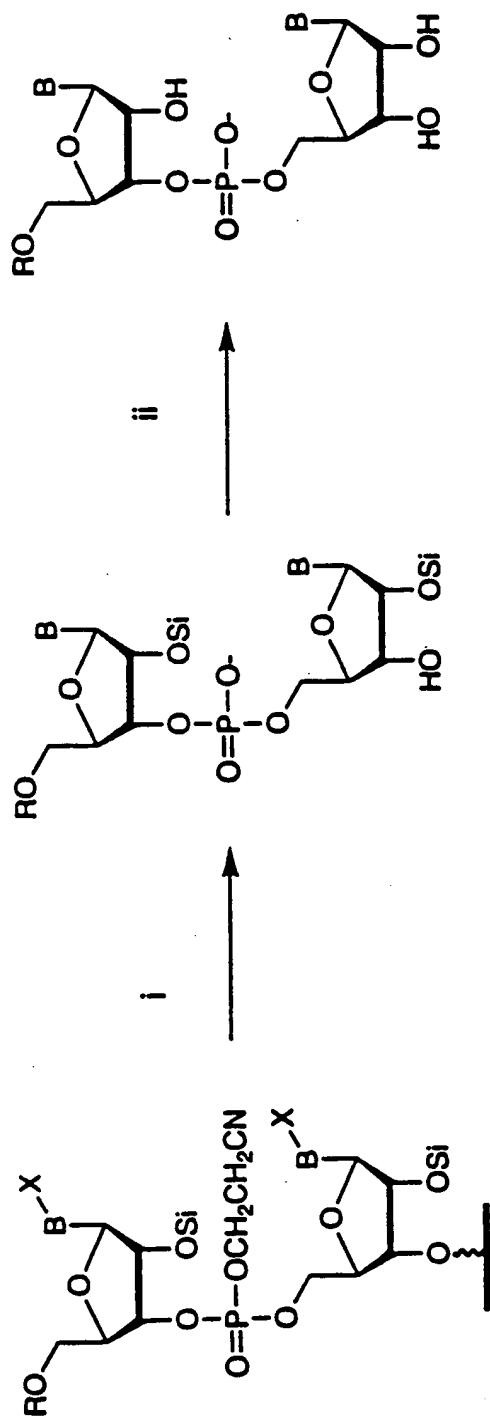


B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

SEM = (trimethylsilyl)ethoxymethyl



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- i) MA or AMA, 30 m @ 65 °C, or $\text{NH}_4\text{OH}/\text{EtOH}$,
- ii) 8-16h @ 55-65°C
- iii) Anhydrous TEA•HF, 30 m @ 65 °C or TBAF, 6-24h @ R.T.
- iv) H^+

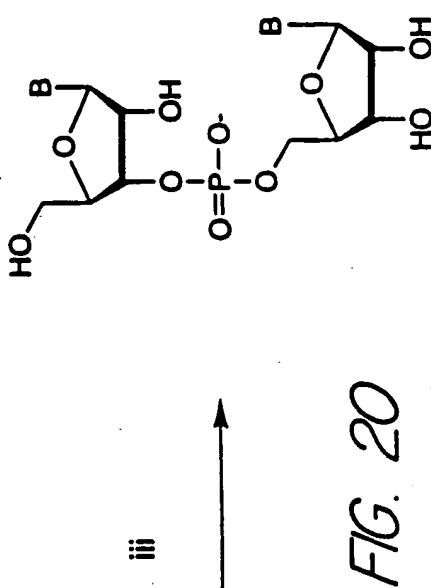
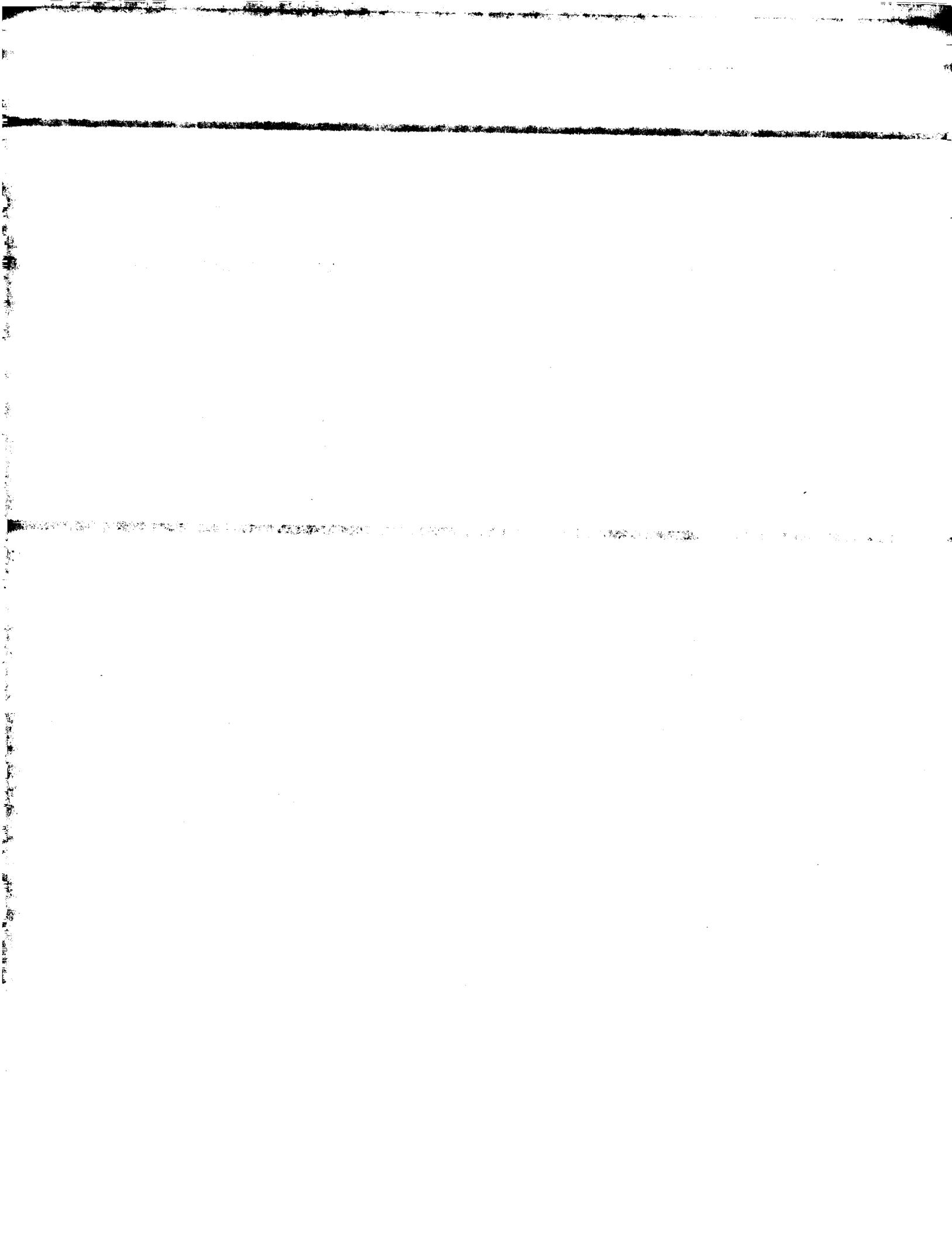


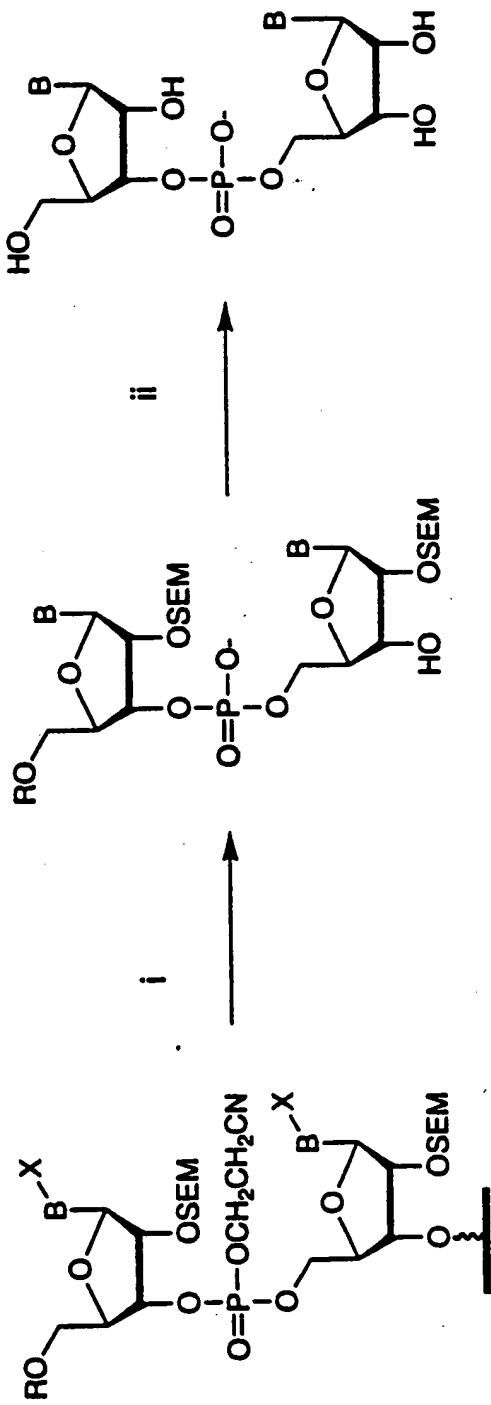
FIG. 20

$\text{R} = \text{H}$ or DMT or other hydroxyl protection
 $\text{X} = \text{Exocyclic amino group protection}$



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FIG. 21.



i) MA or AMA, 30 m @ 65 °C or NH₄OH/EtOH, 8-16h @ 55-65 °C

ii) BF₃•OEt₂

SEM = (trimethylsilyl)ethoxymethyl

R = H or DMT or other hydroxyl protection

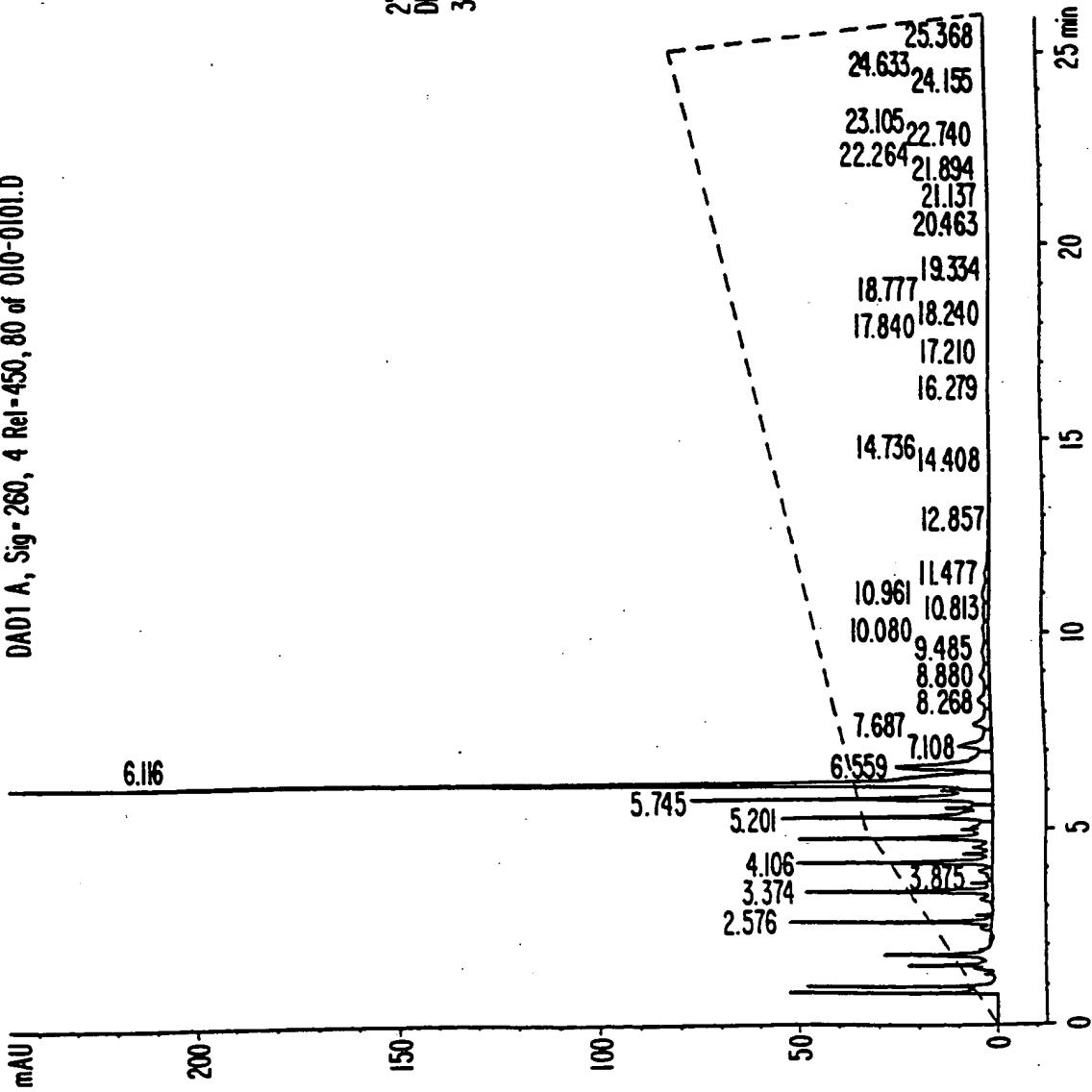
X = Exocyclic amino group protection

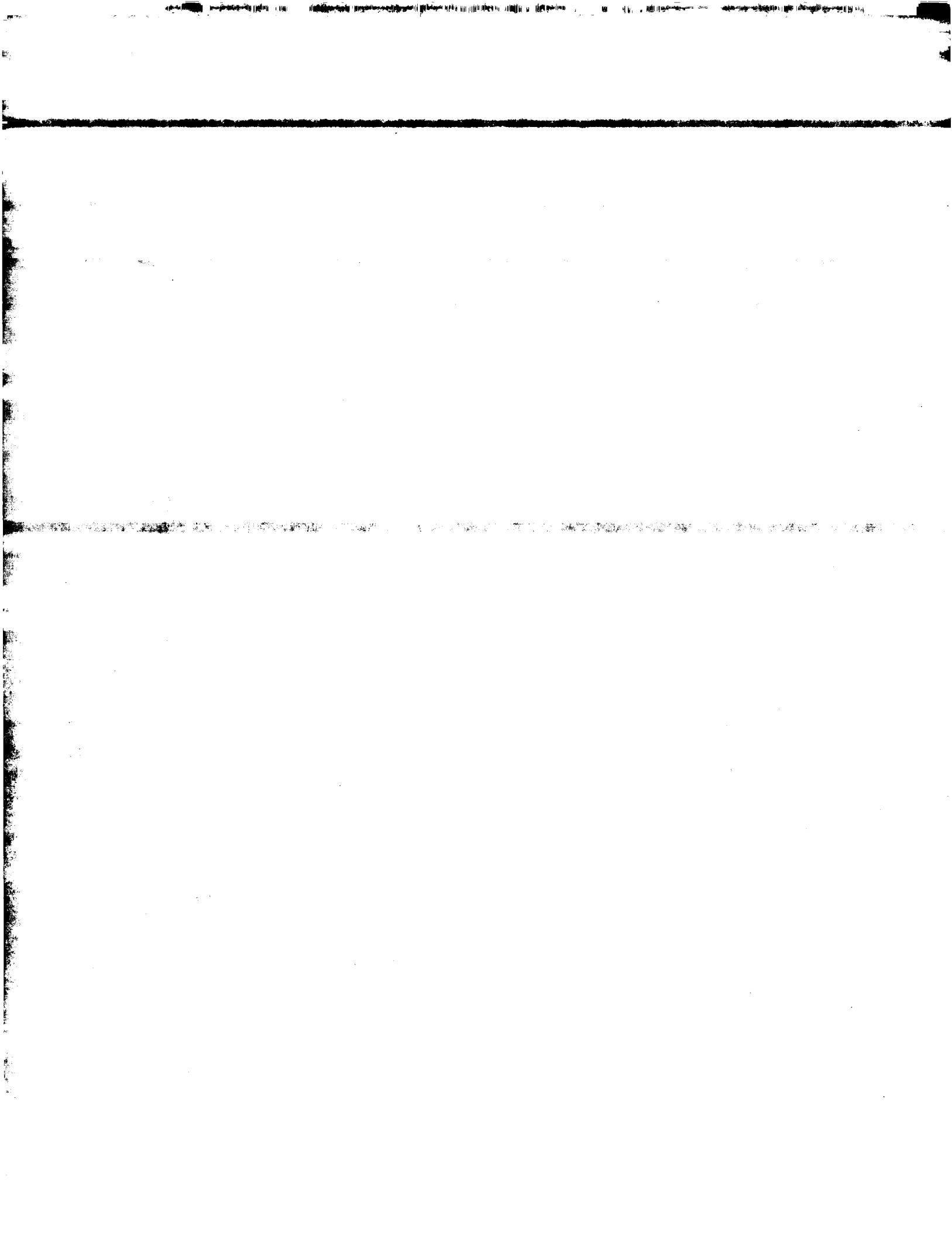
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DAD1 A, Sig. 260, 4 Rel-450, 80 of 010-0101.0

FIG. 22.

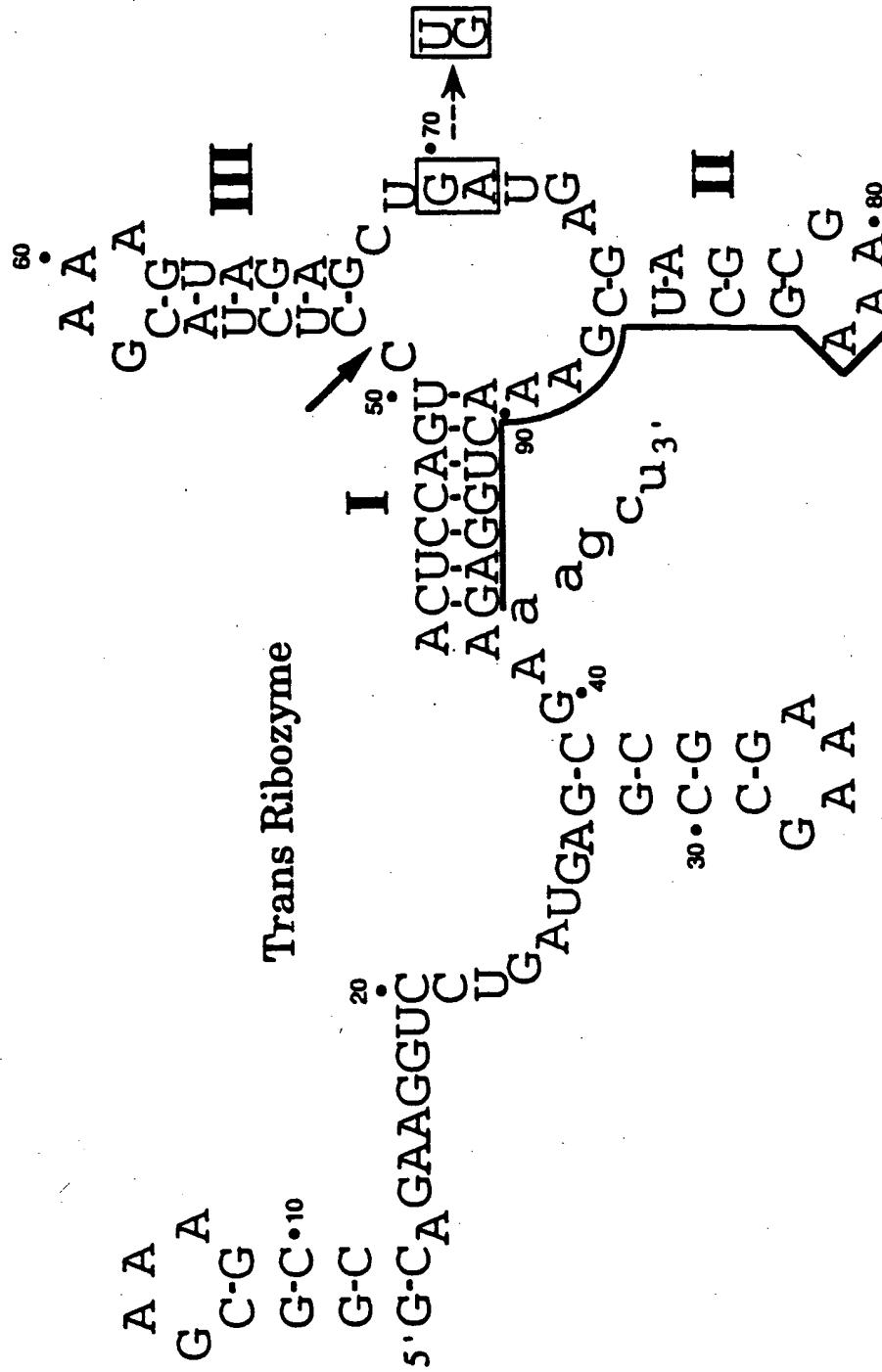
2'-O-SEM PROTECTED U 10-mer
DEPROTECTED WITH $\text{BF}_3 \cdot \text{OEt}_2$
30 m, 3 eq./nucleotide



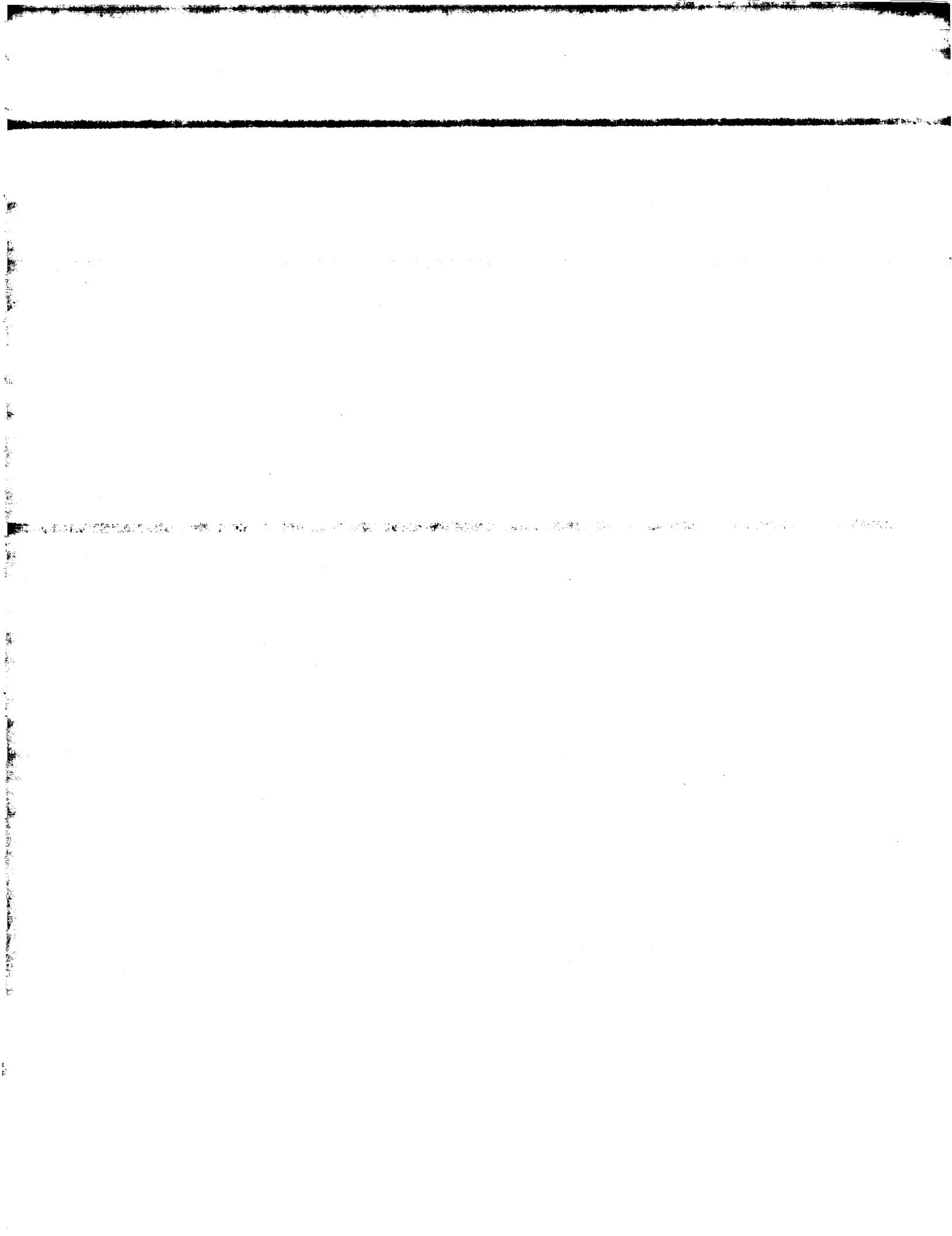


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FIG. 23.



3' Cis-acting Ribozyme



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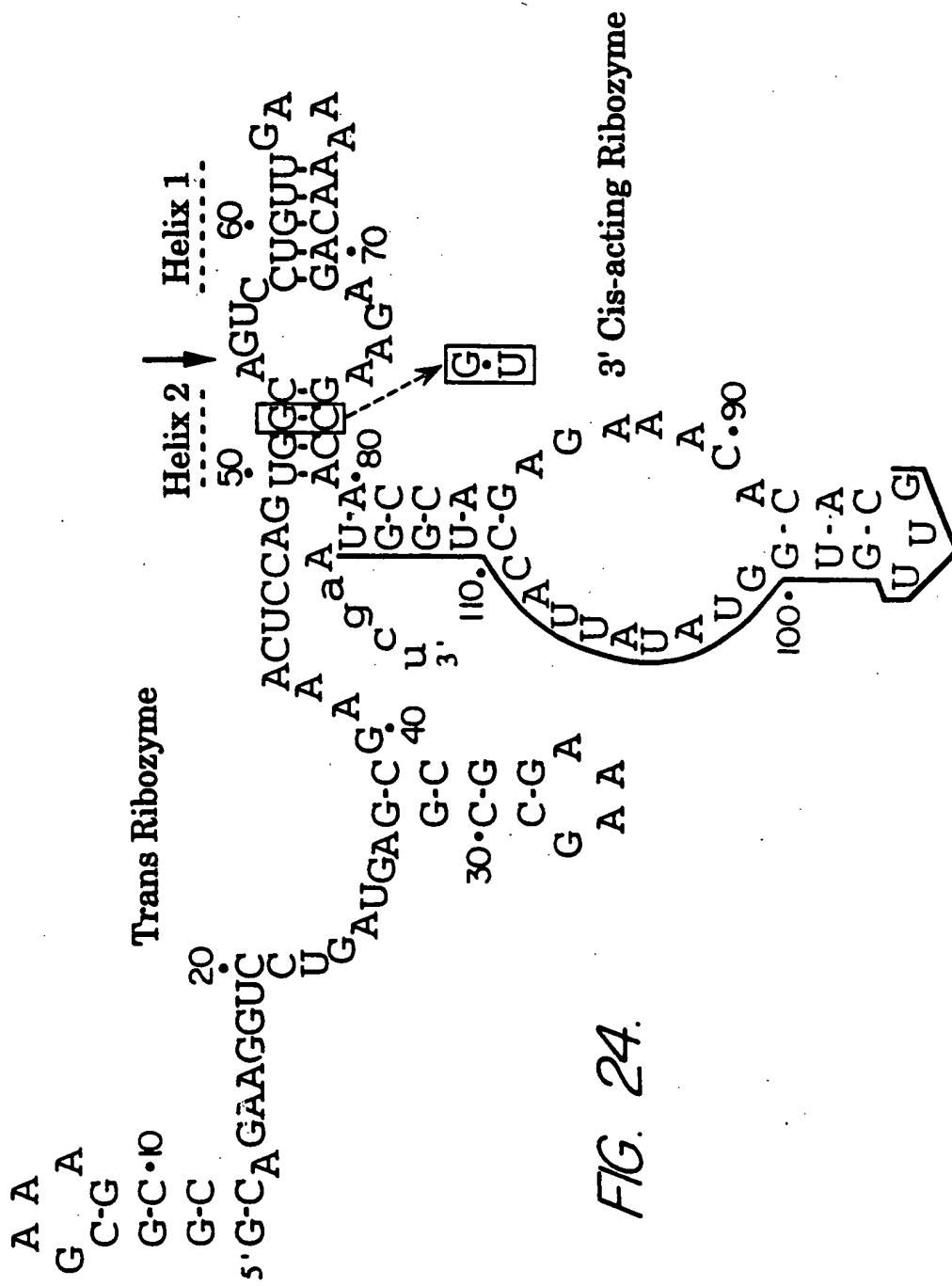
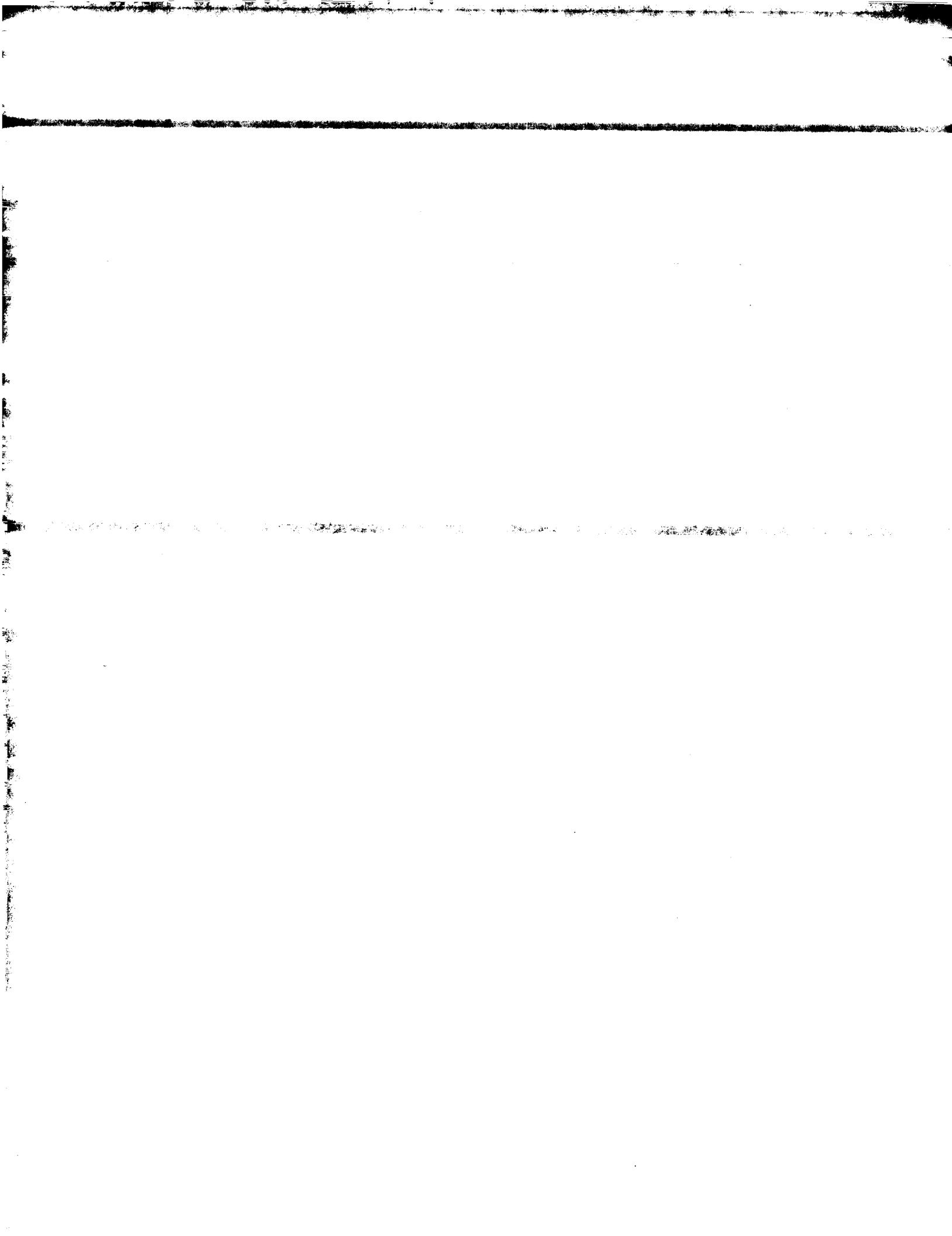


FIG. 24.



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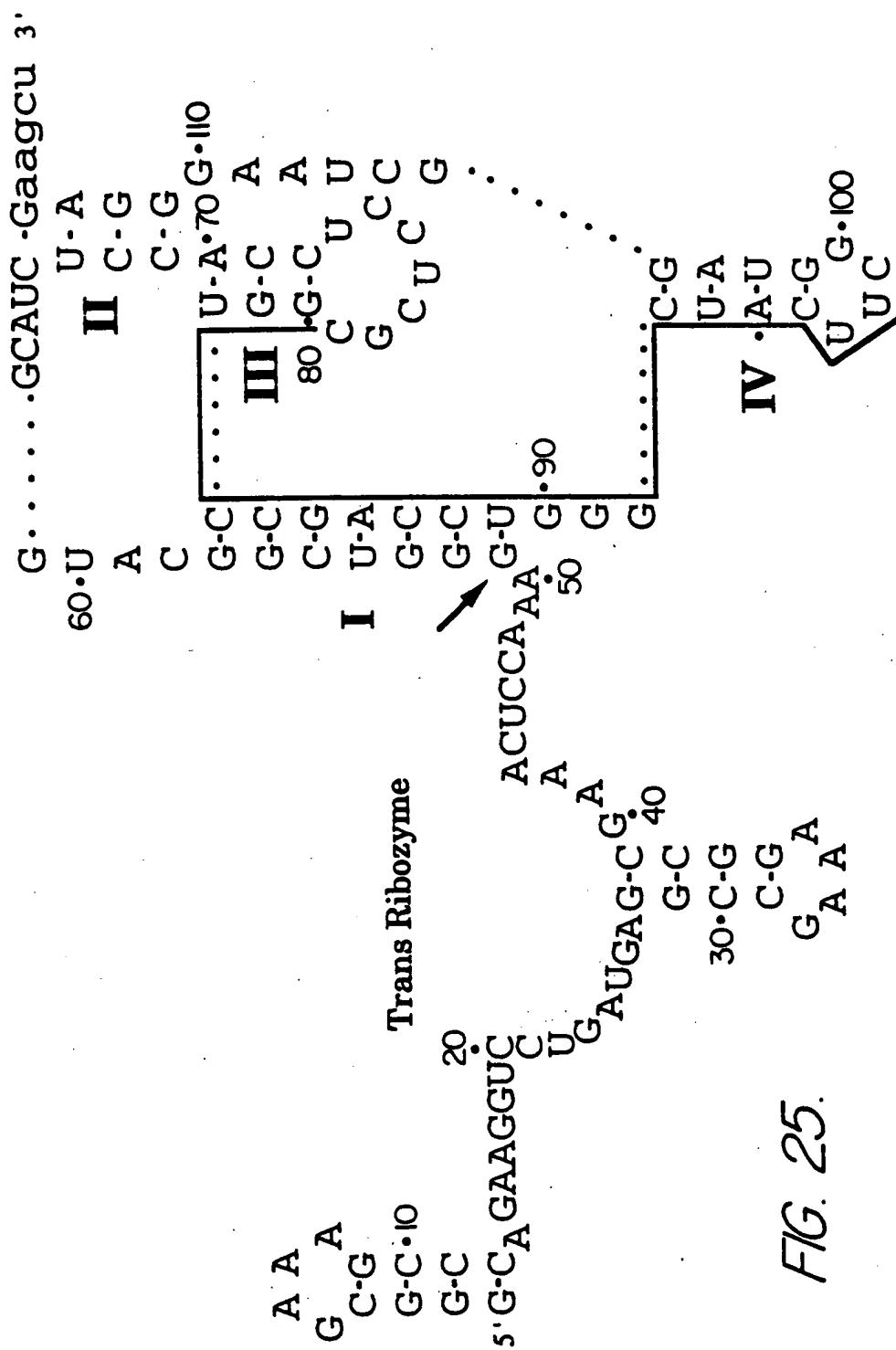
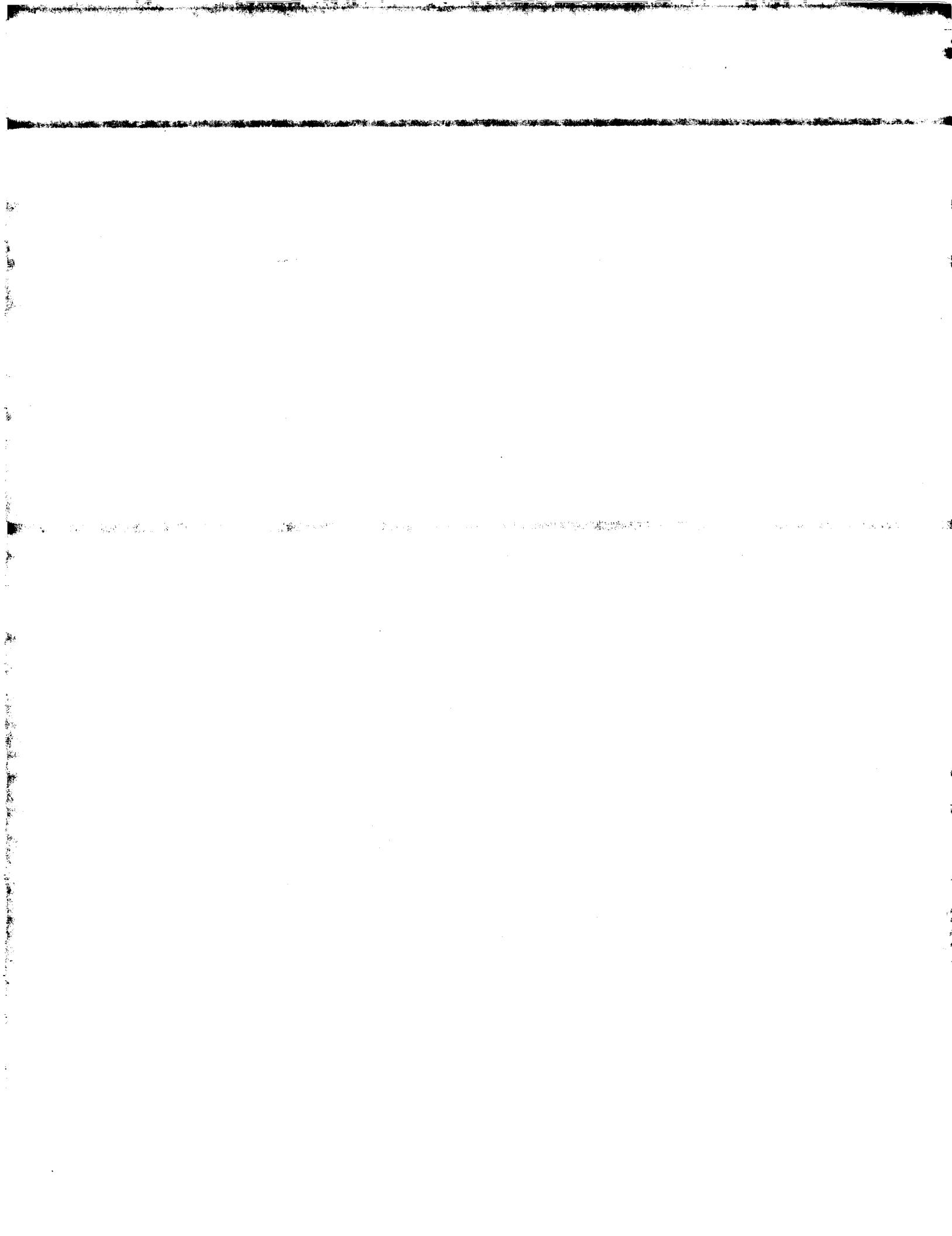


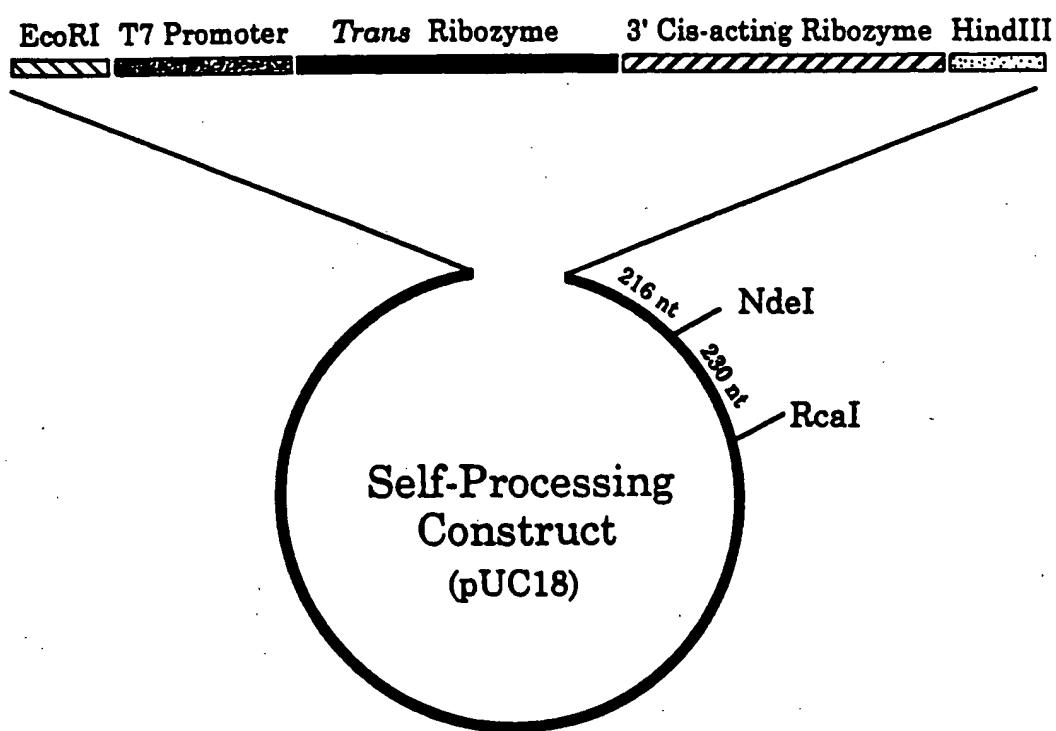
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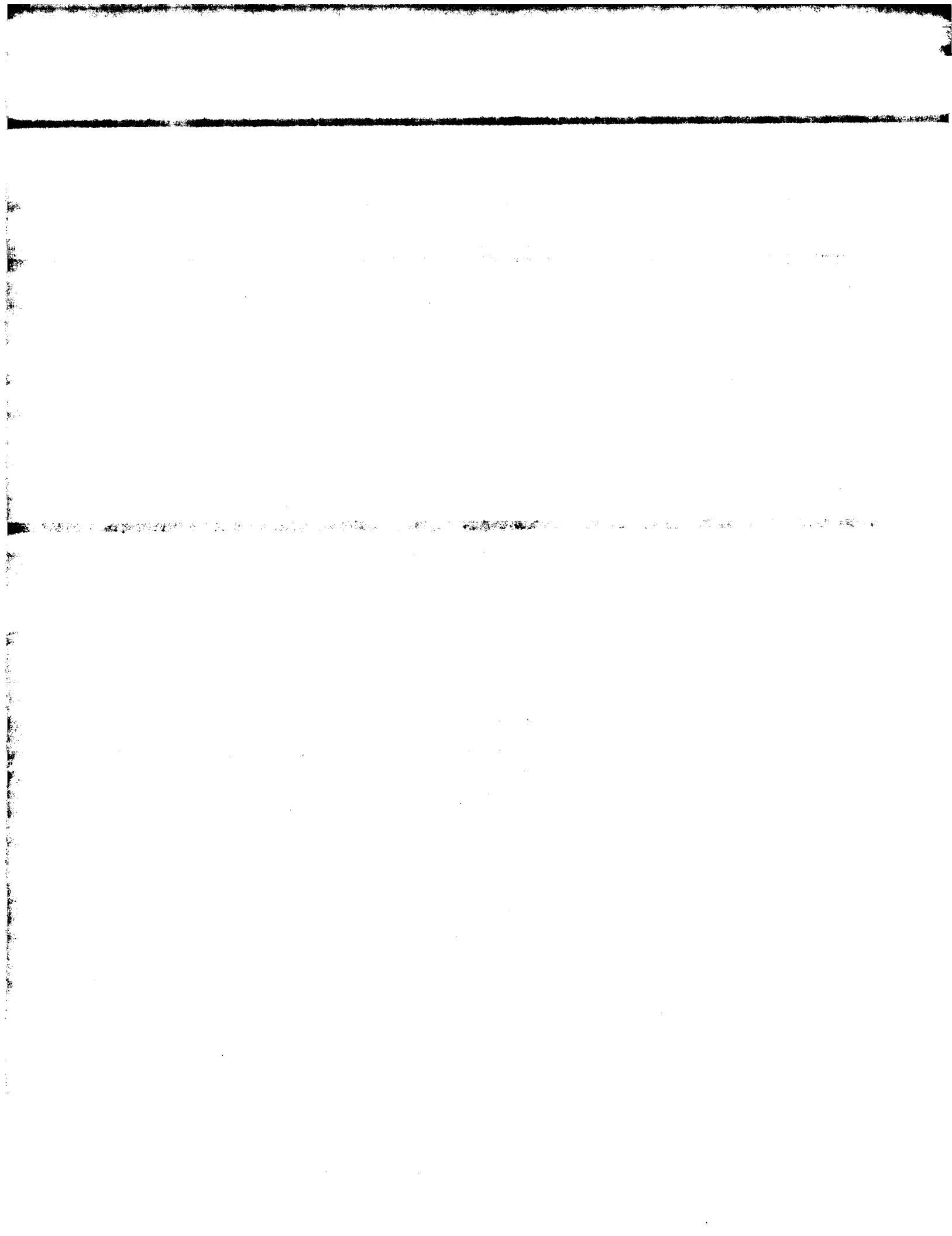
SUBSTITUTE SHEET (RULE 26)



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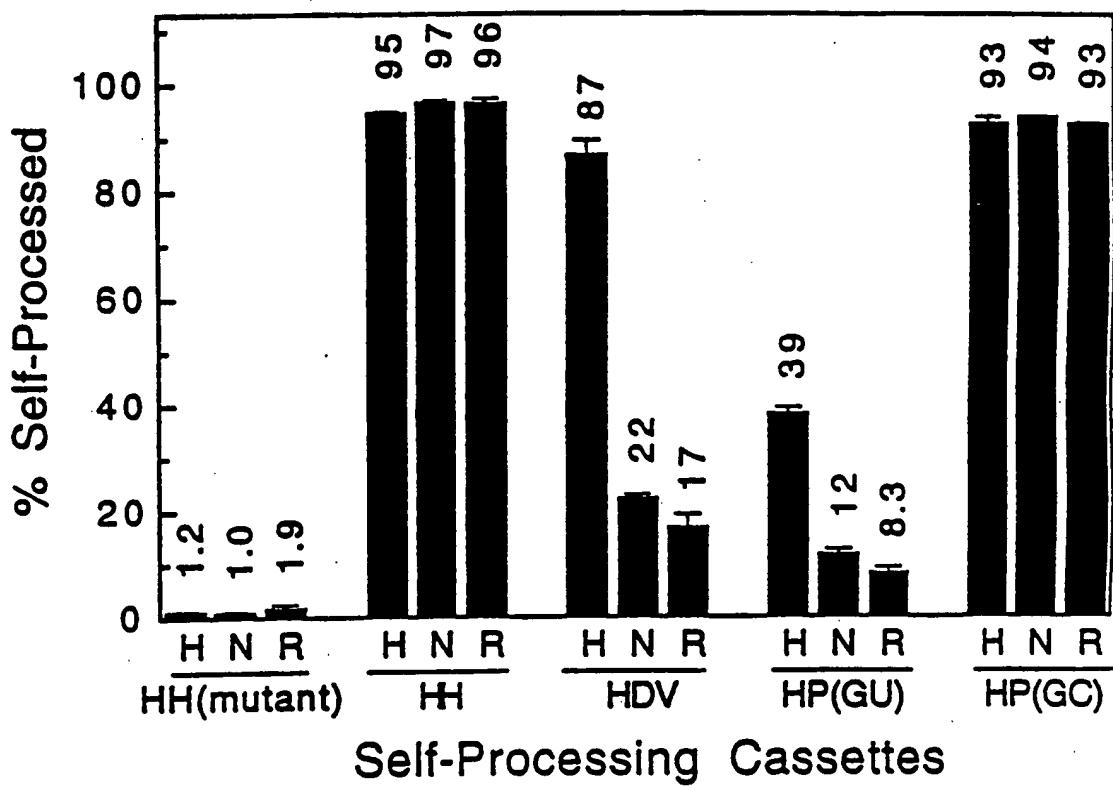
FIG. 26.





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FIG. 27.



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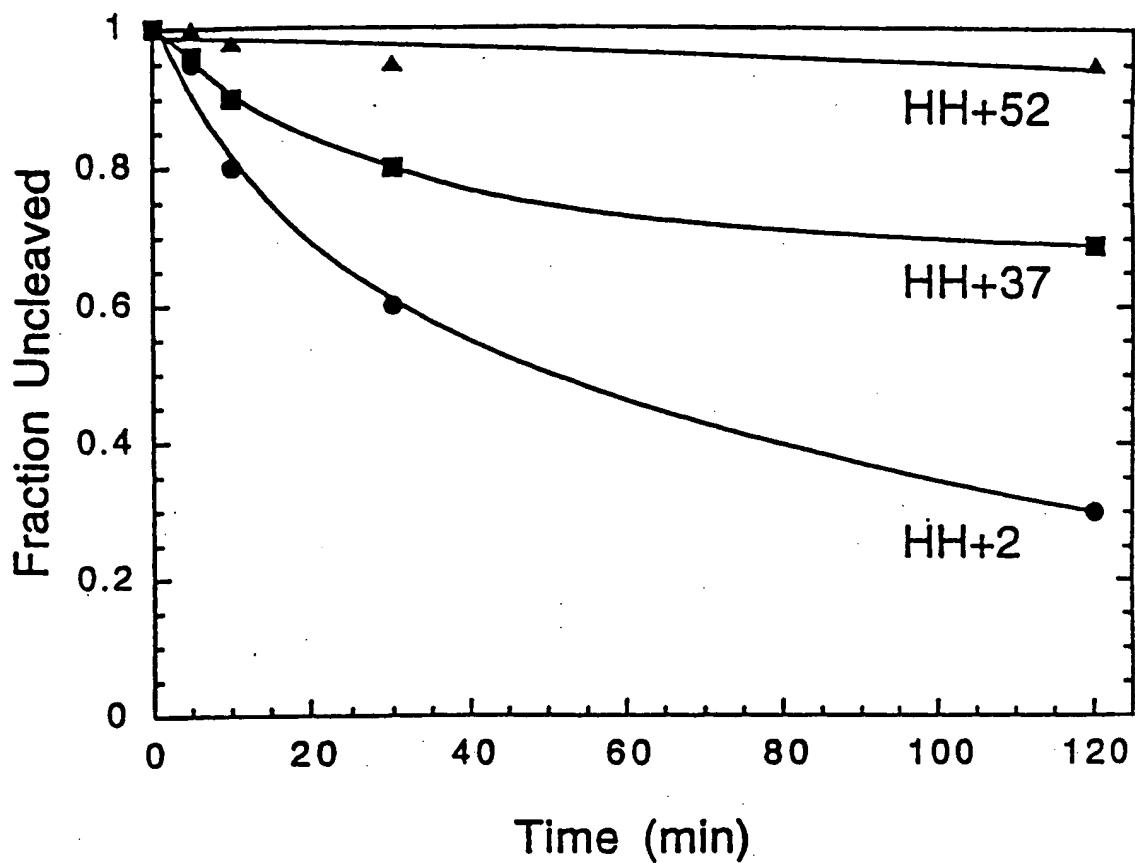
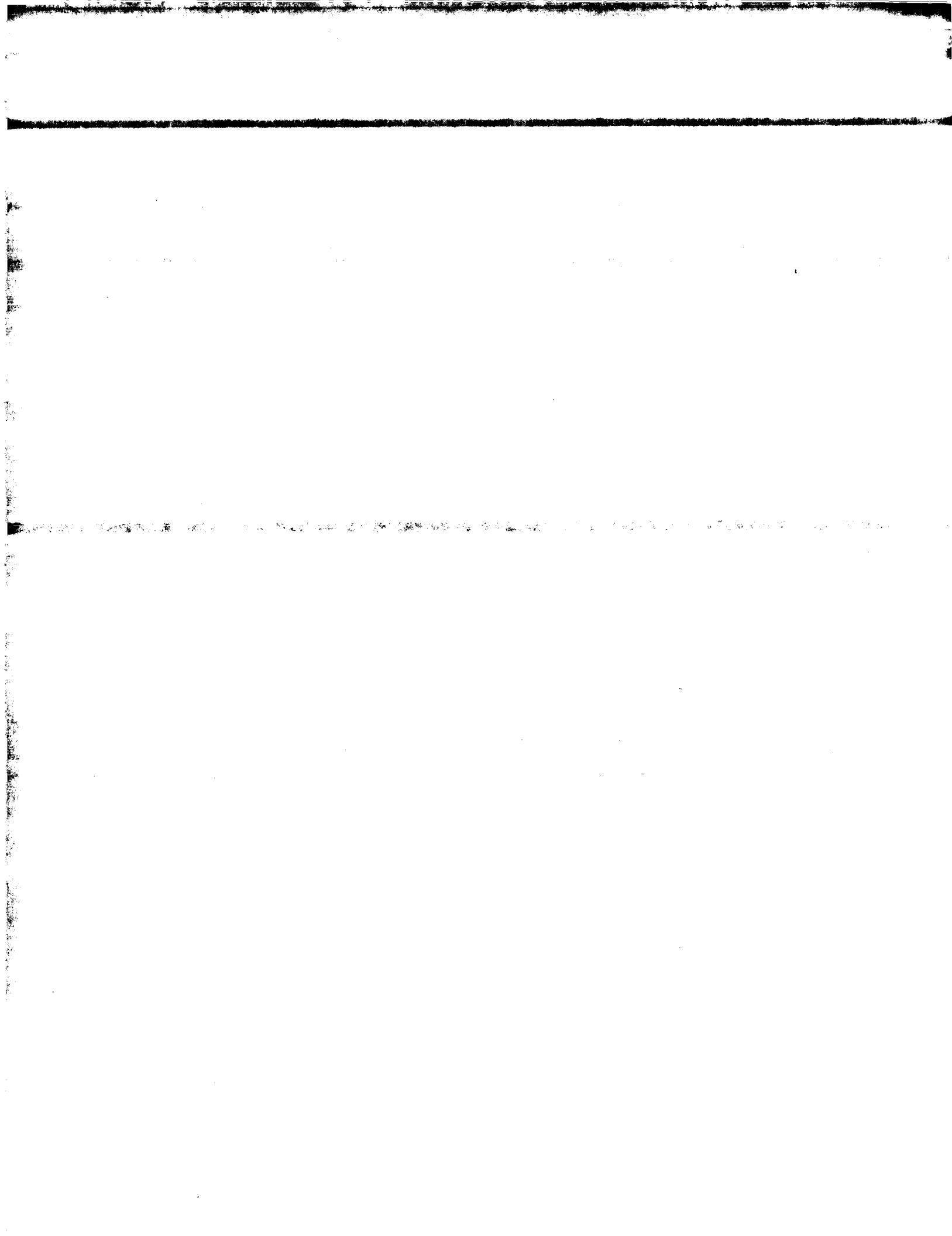
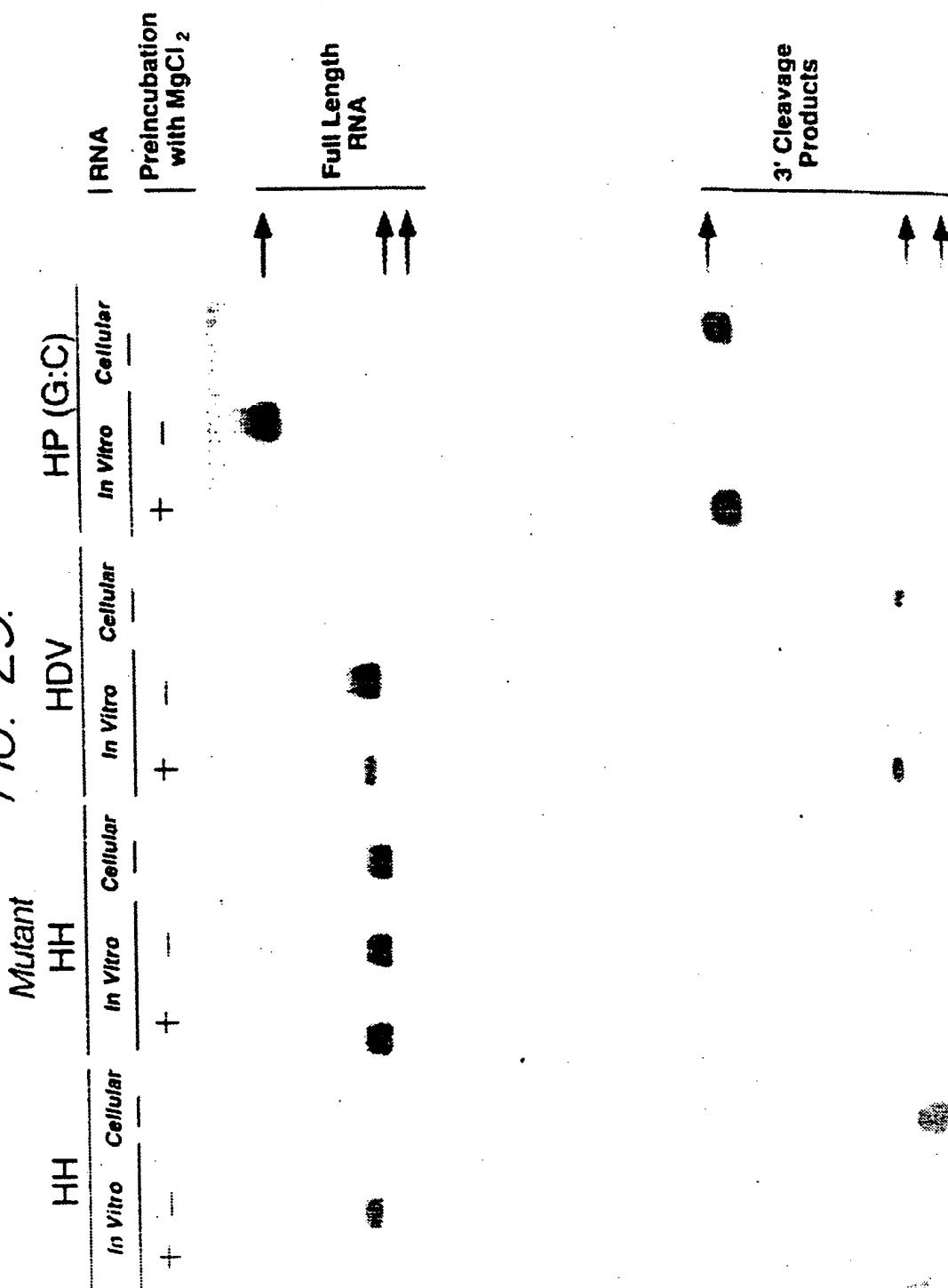


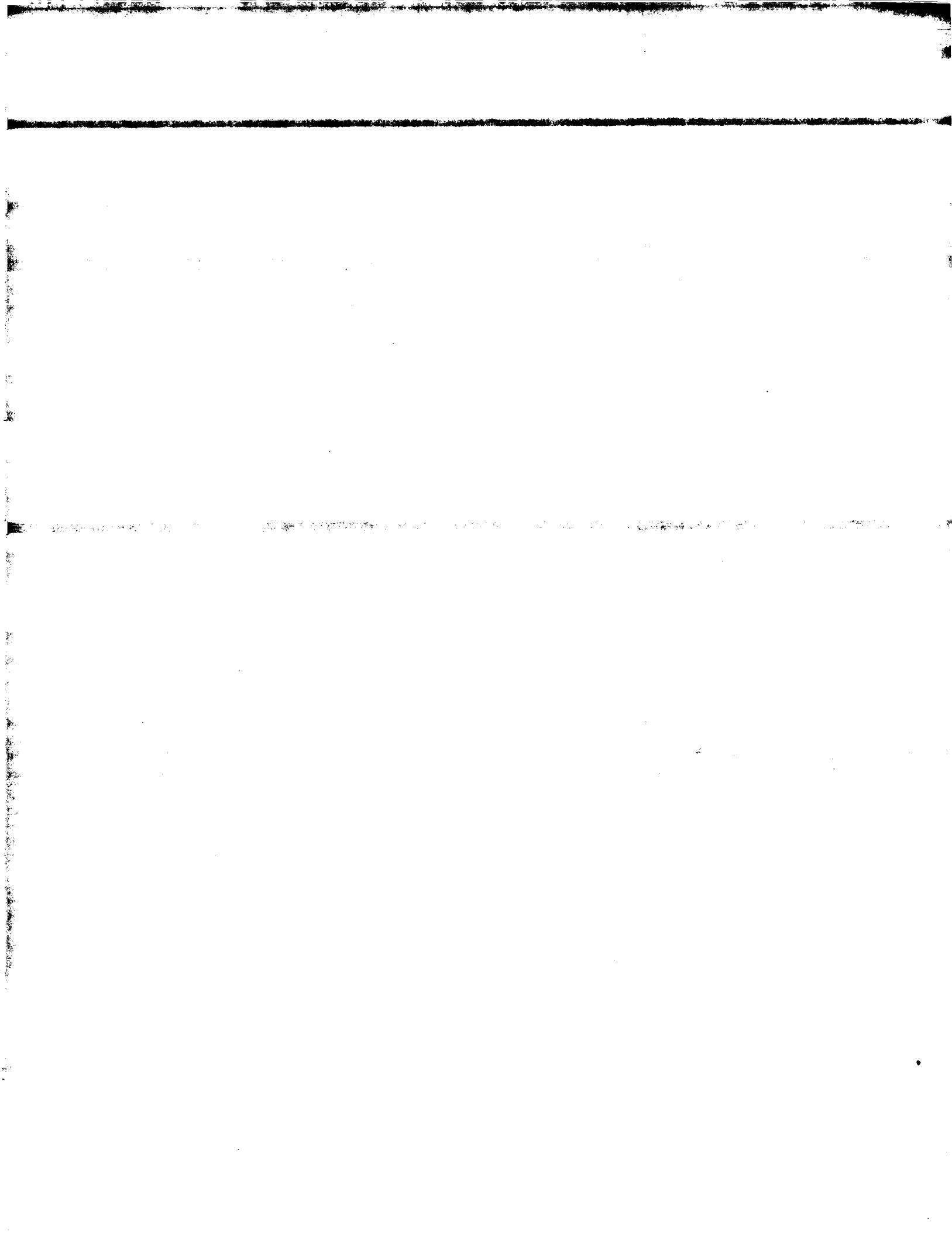
FIG. 28.



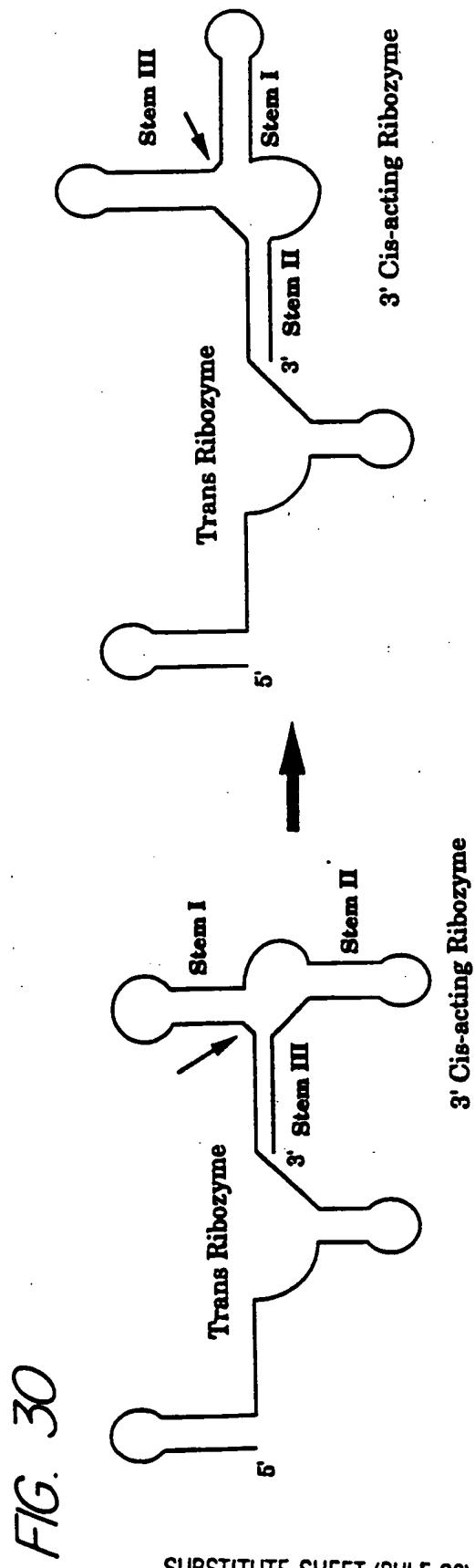
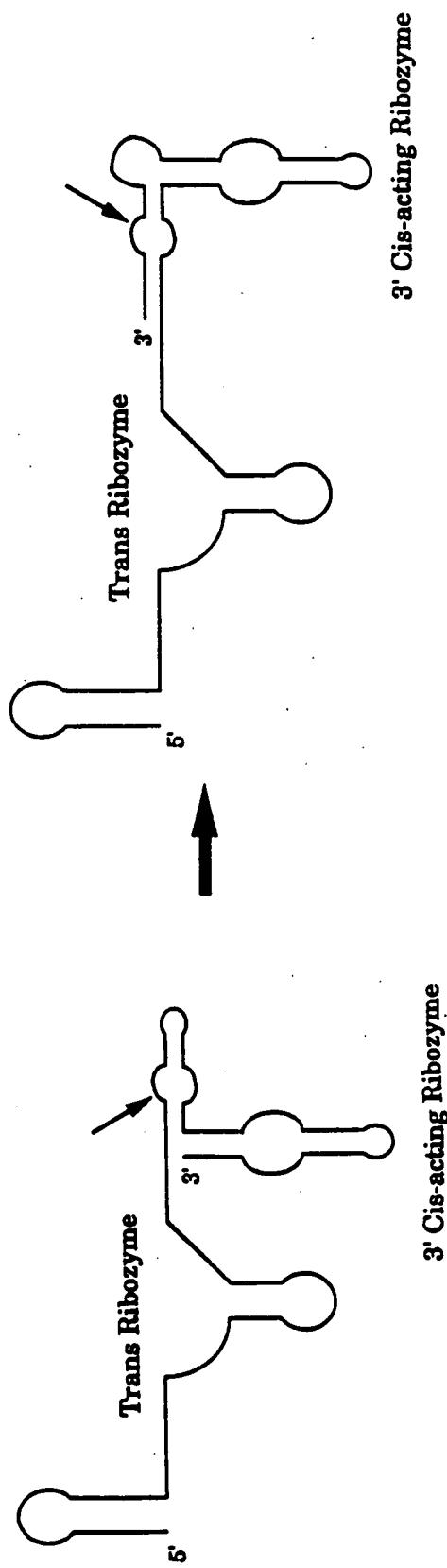
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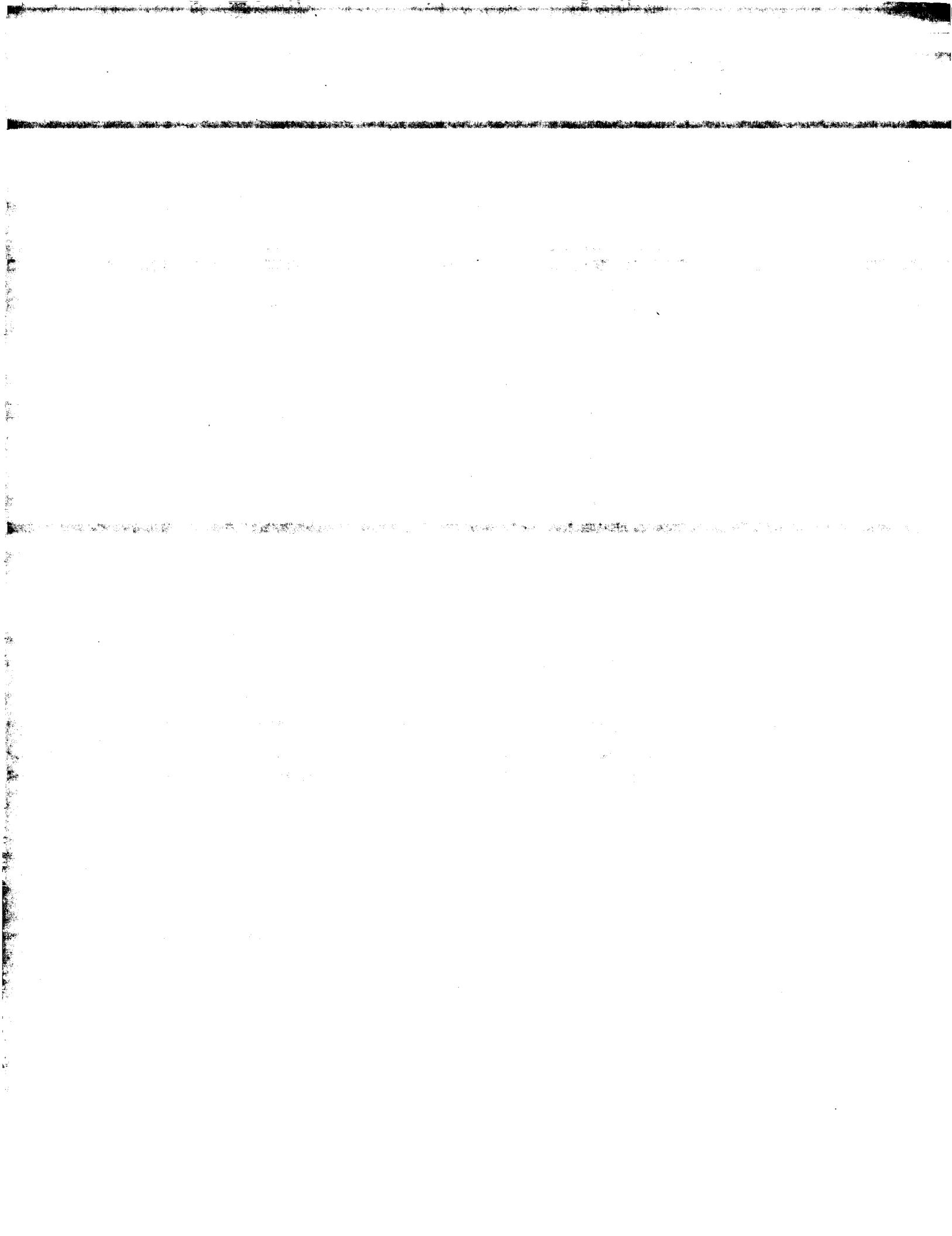
FIG. 29.





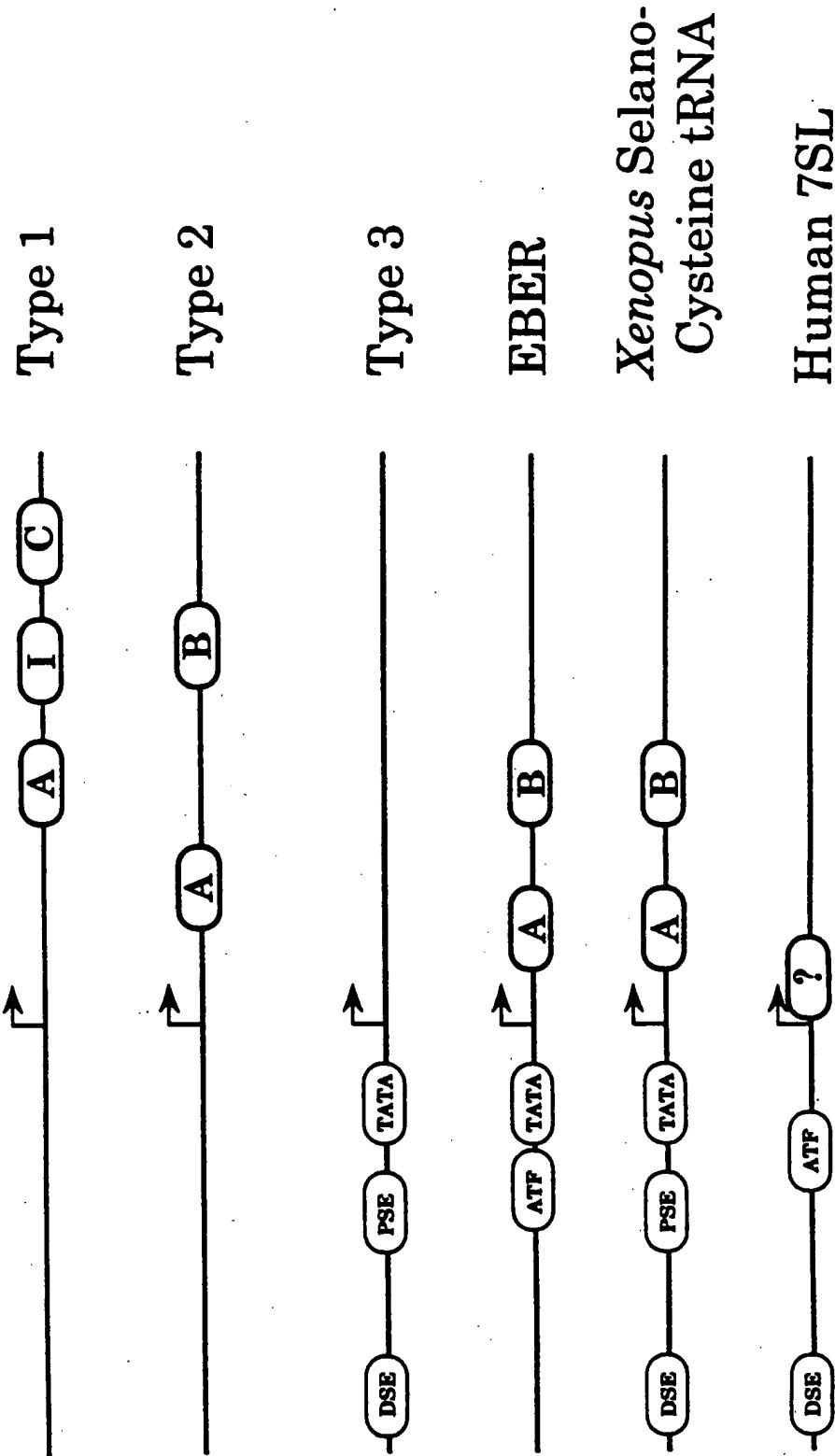
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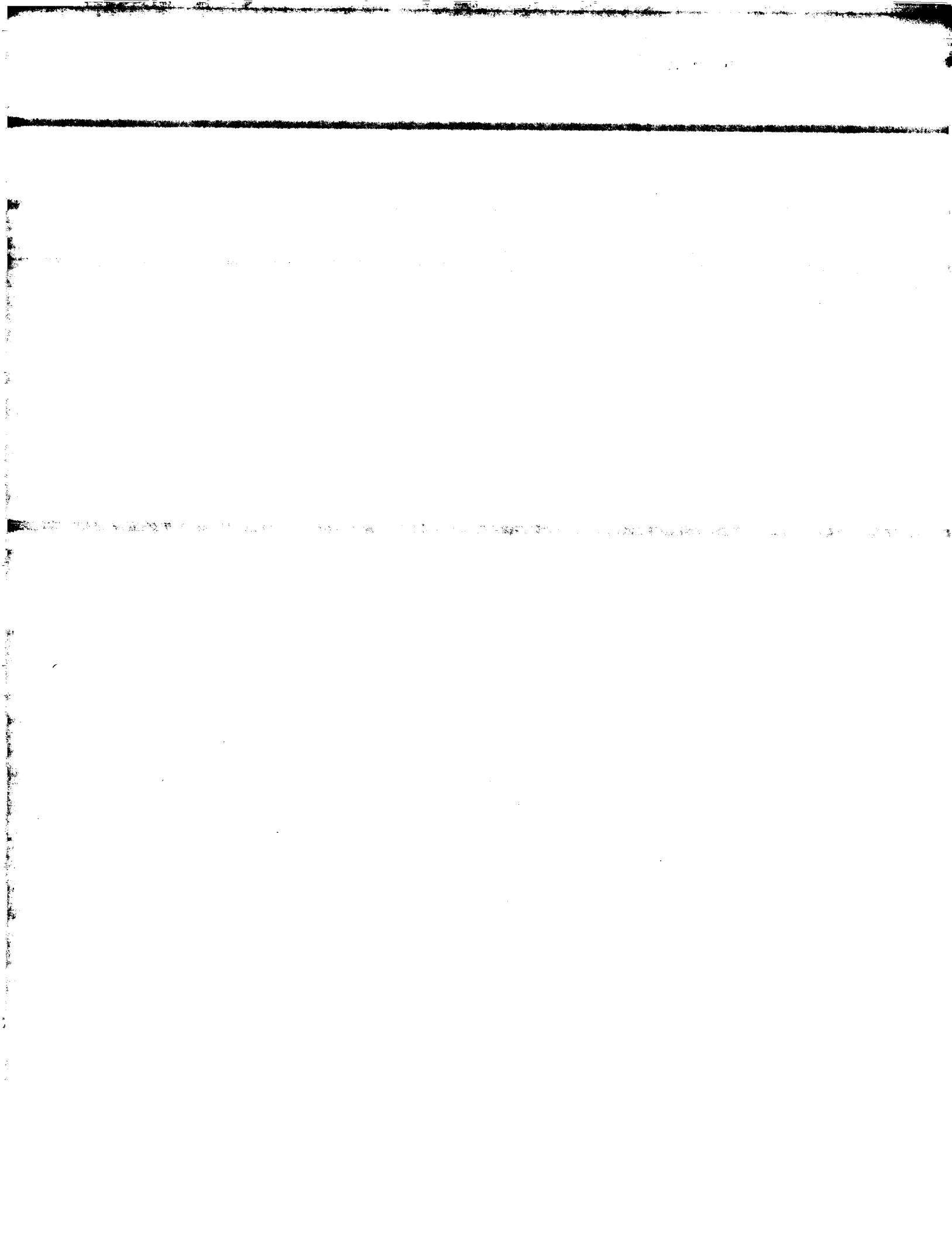
*FIG. 31.*



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FIG. 32.





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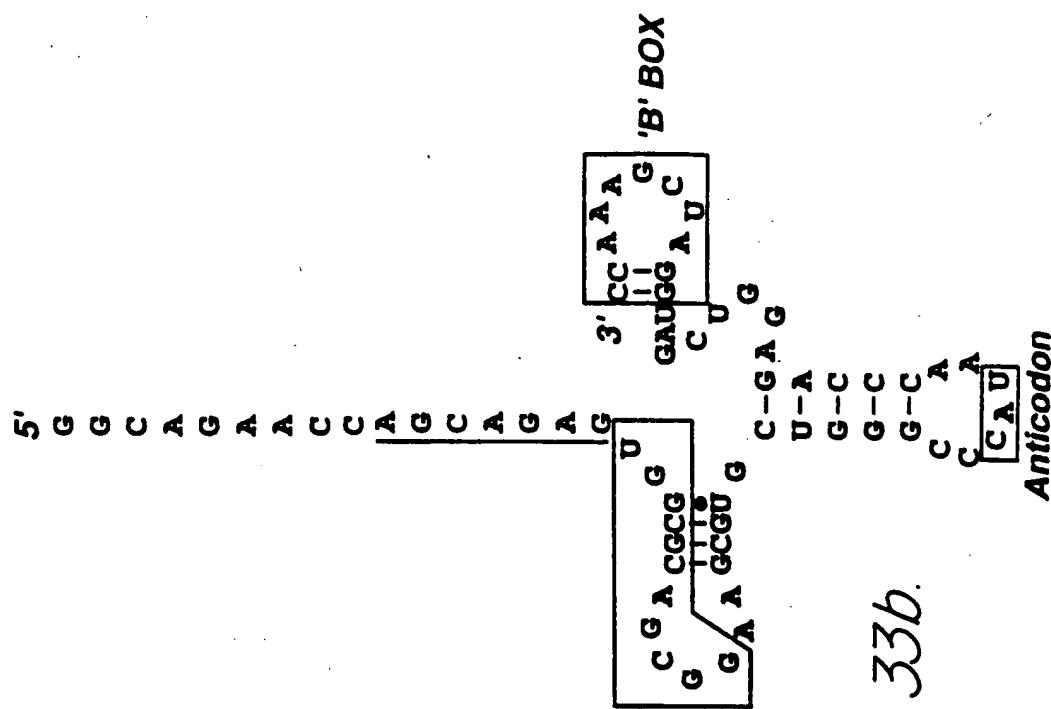
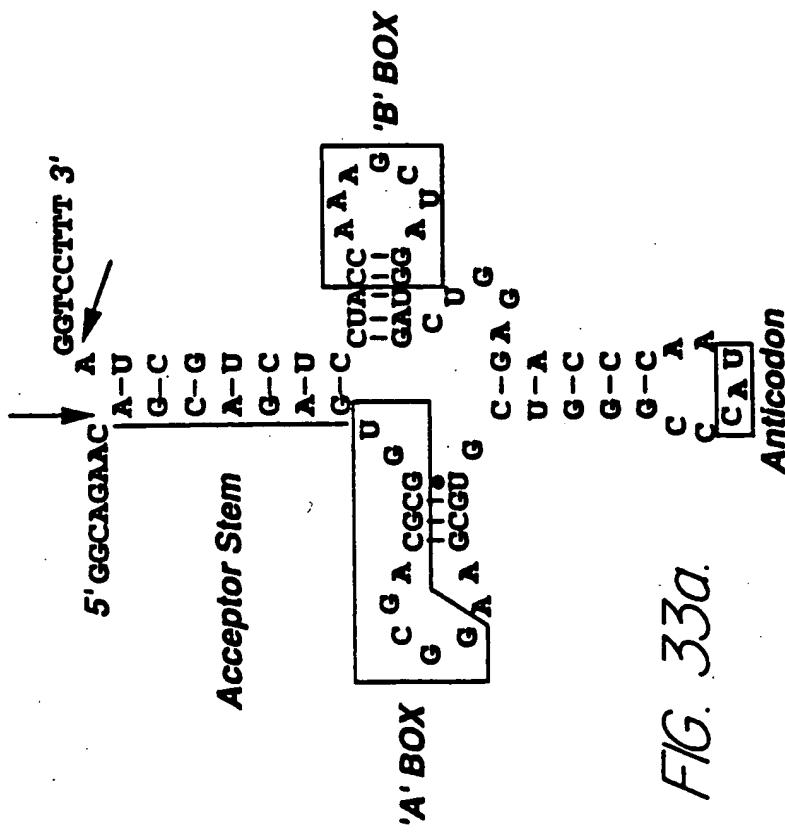


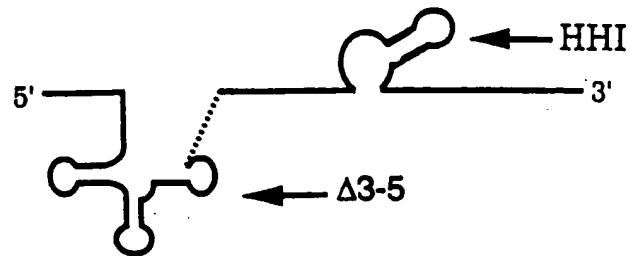
FIG. 33b.

met
tRNA_i

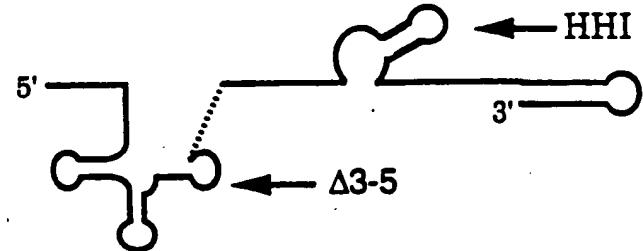


Δ 3-5

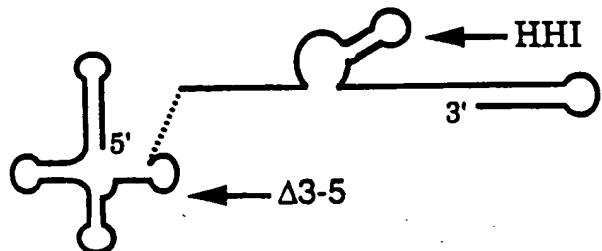
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FIG. 34a. $\Delta 3\text{-}5/\text{HHI}$ *FIG. 34b.*

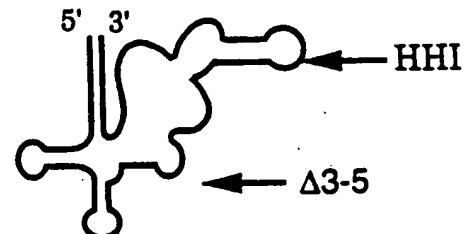
S3

*FIG. 34c.*

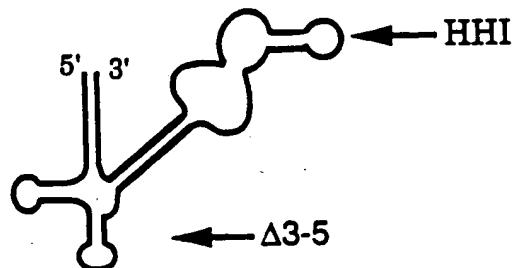
S5

*FIG. 34d.*

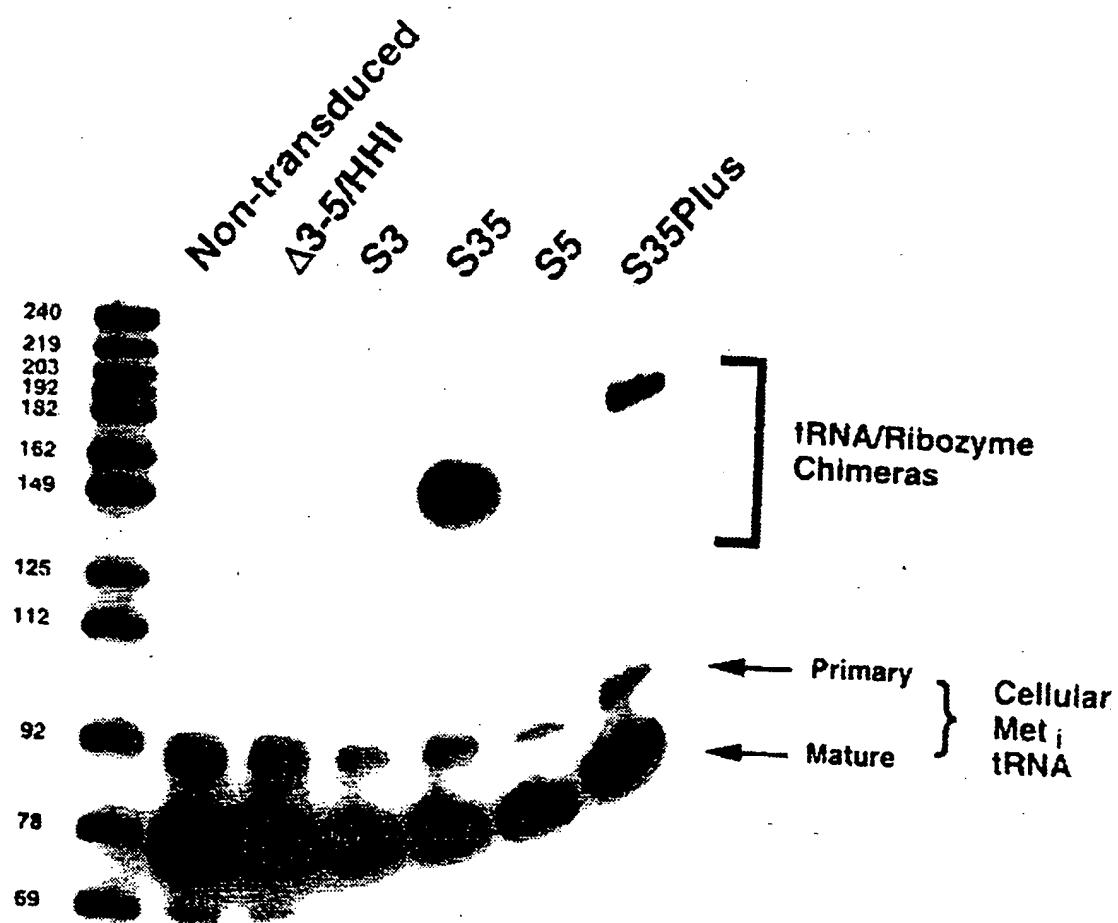
S35

*FIG. 34e.*

S35Plus

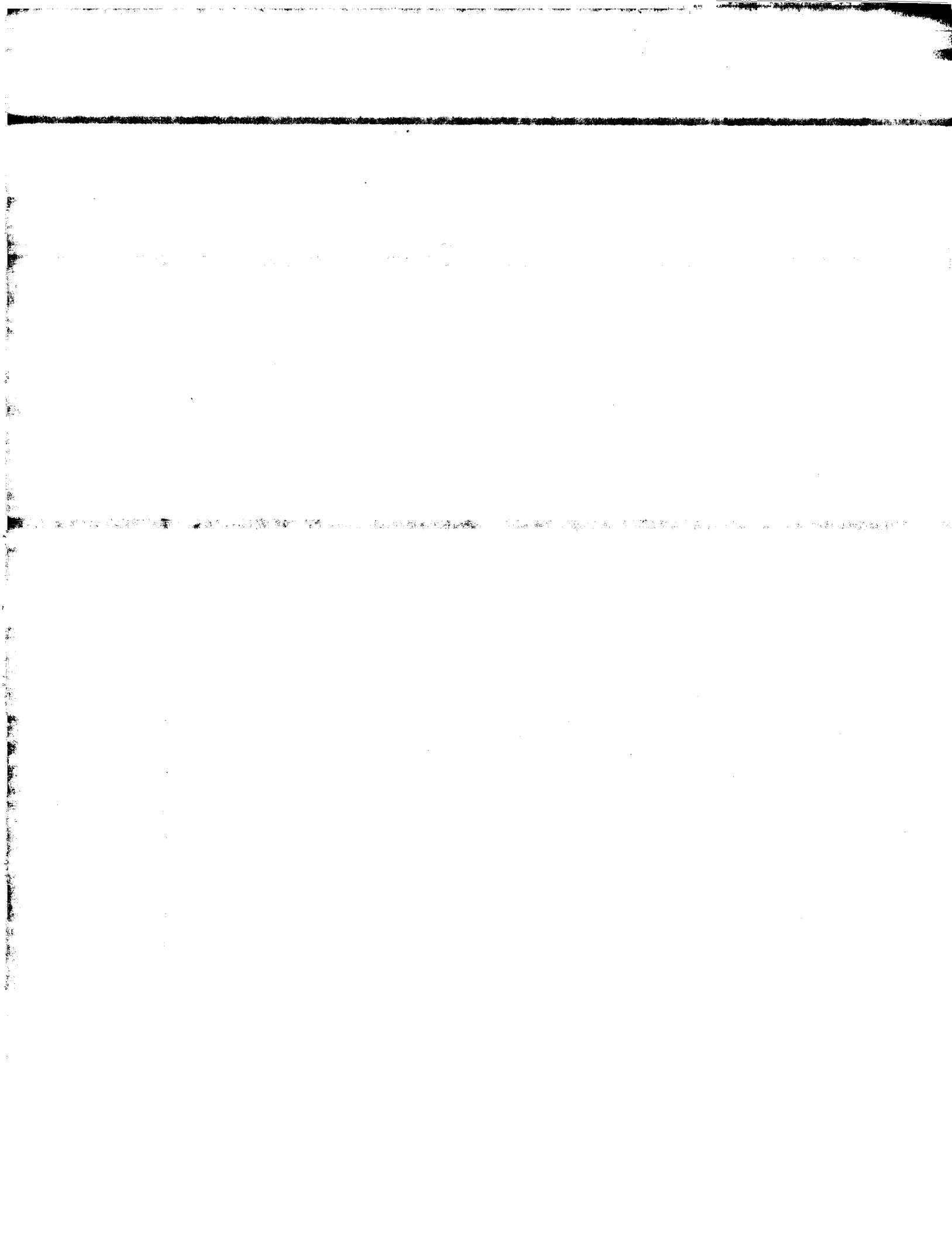


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FIG. 35.



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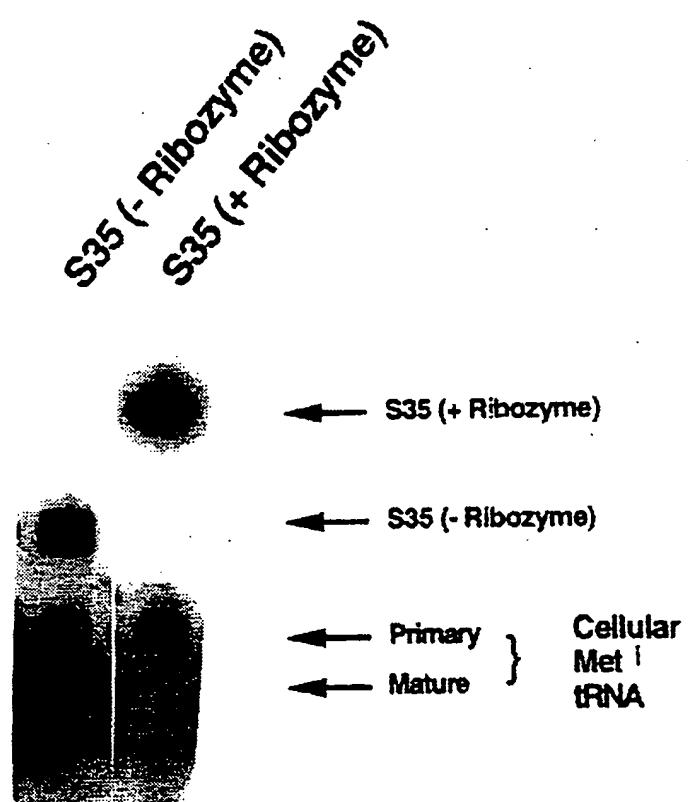
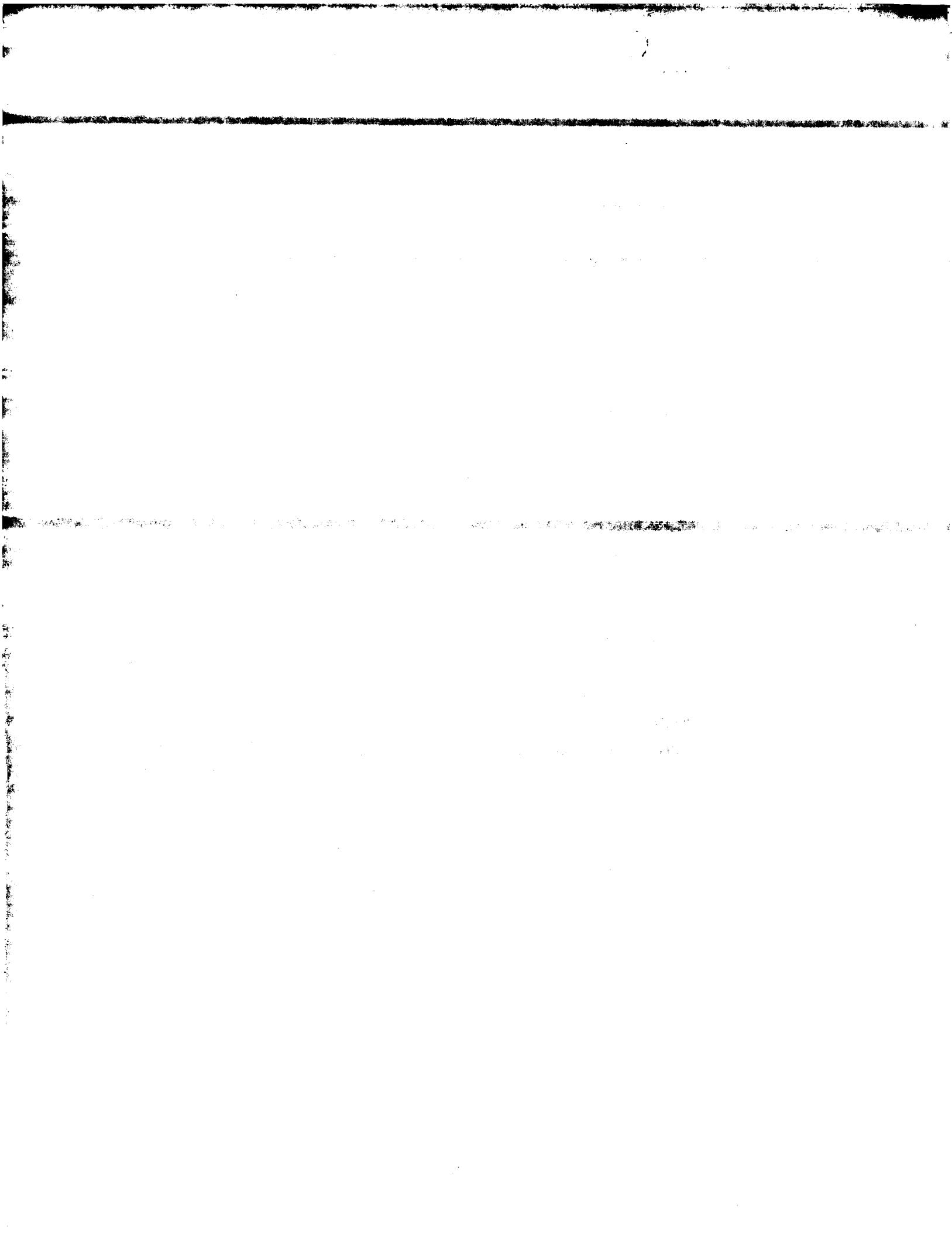


FIG. 36.



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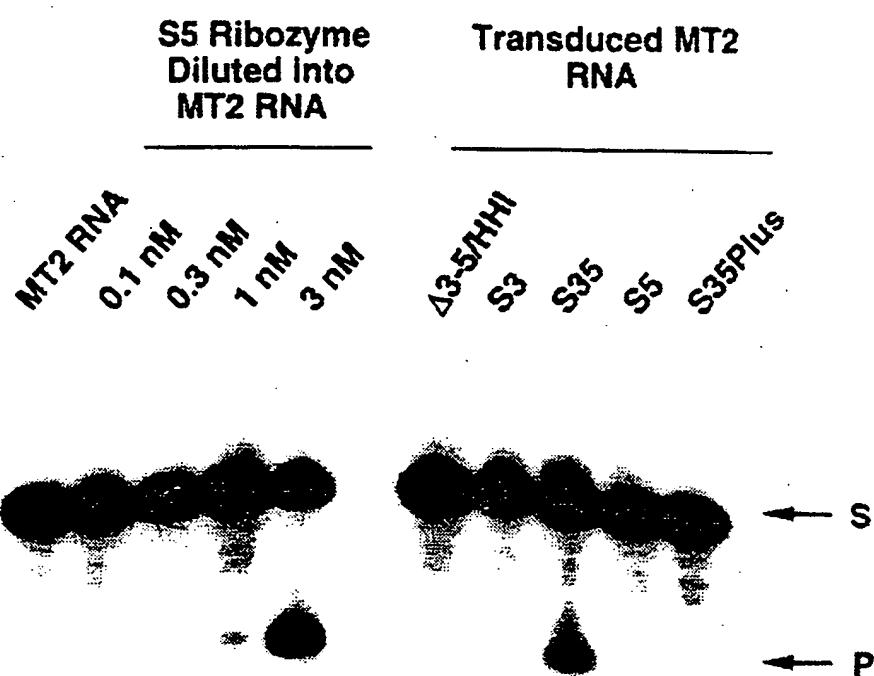
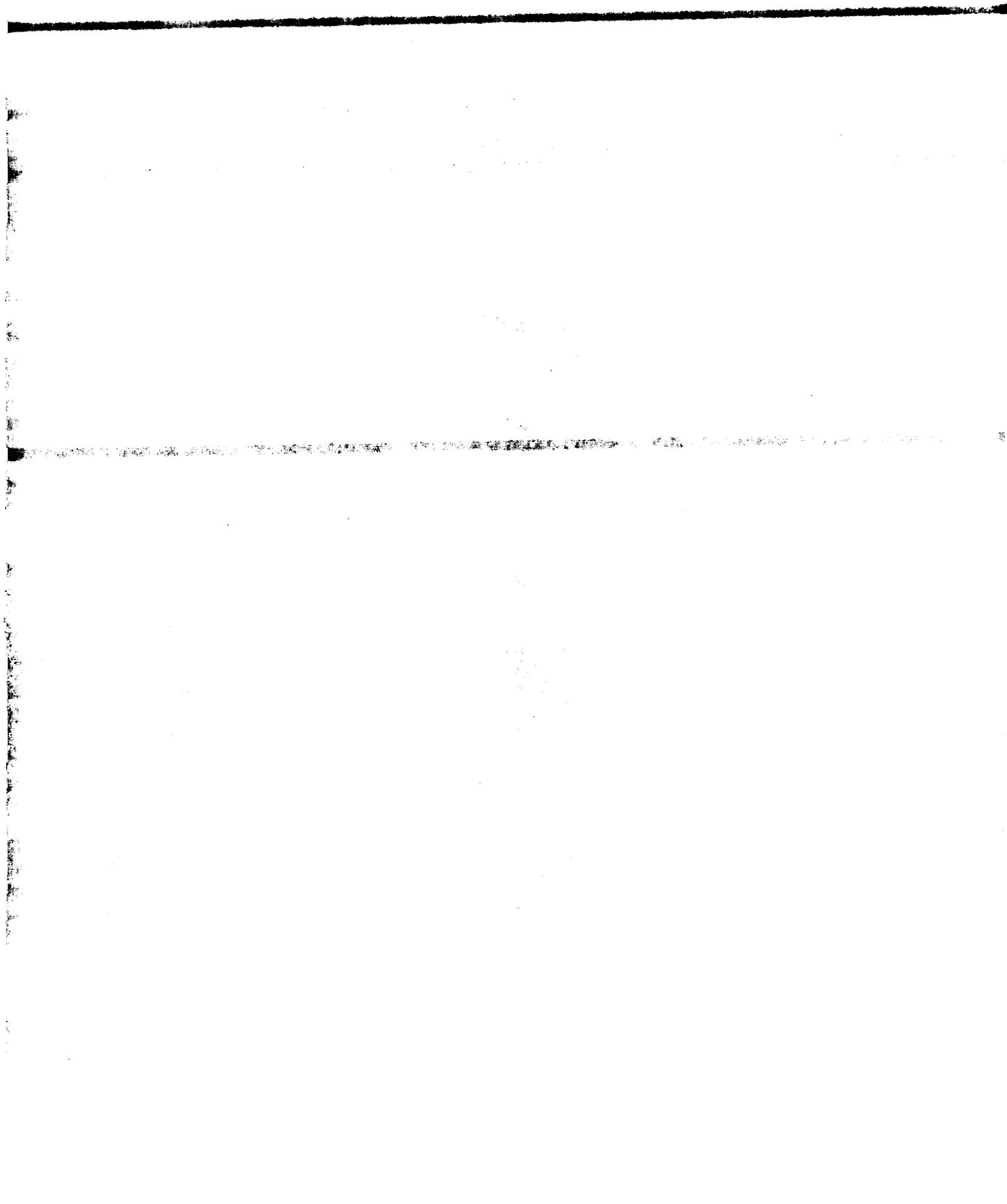
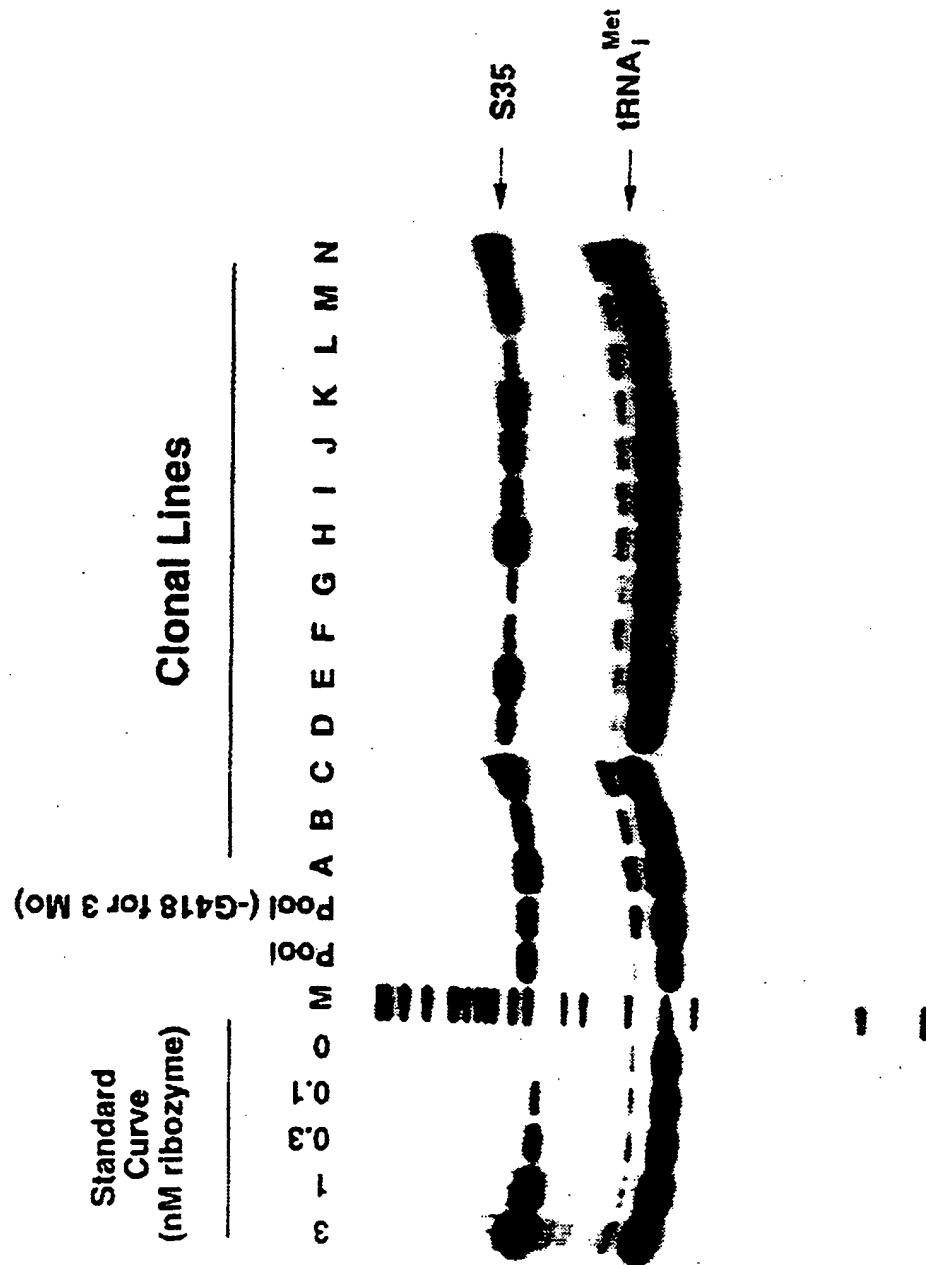


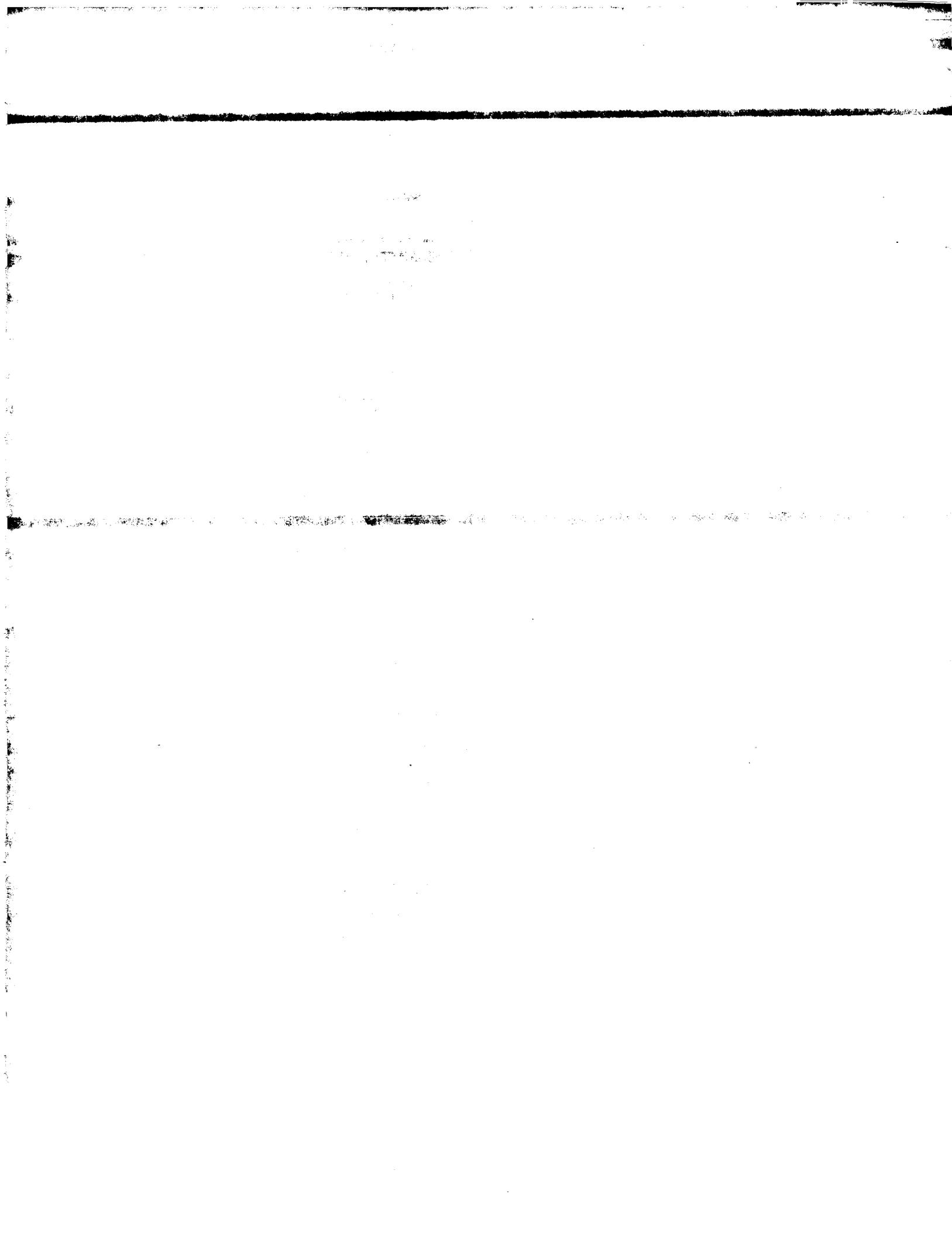
FIG. 37.



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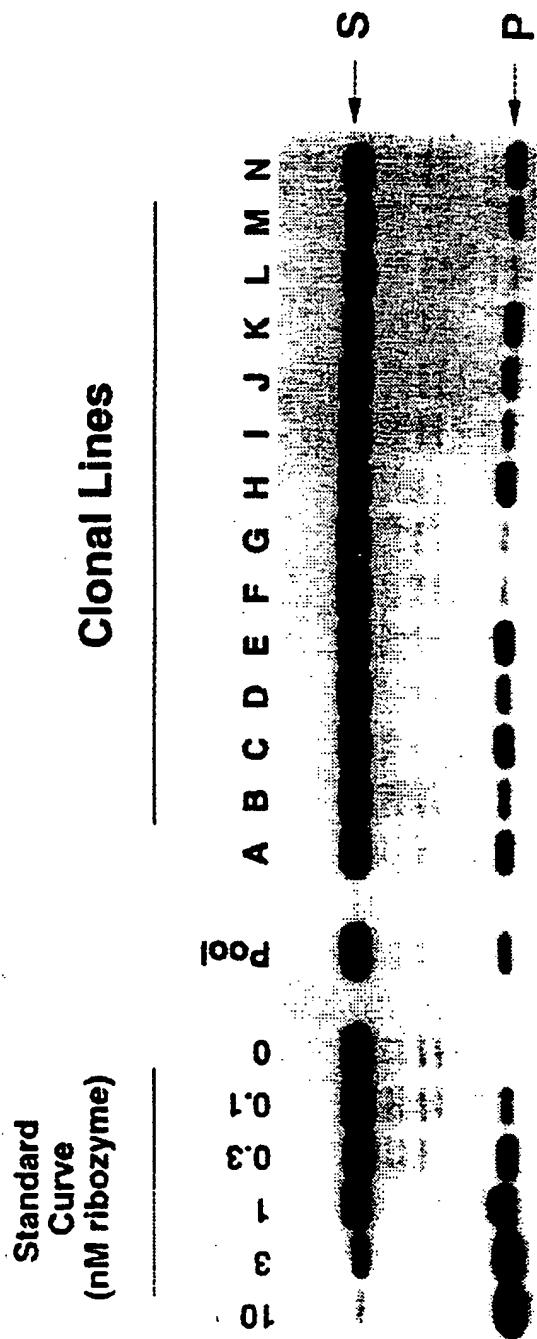
FIG. 38.

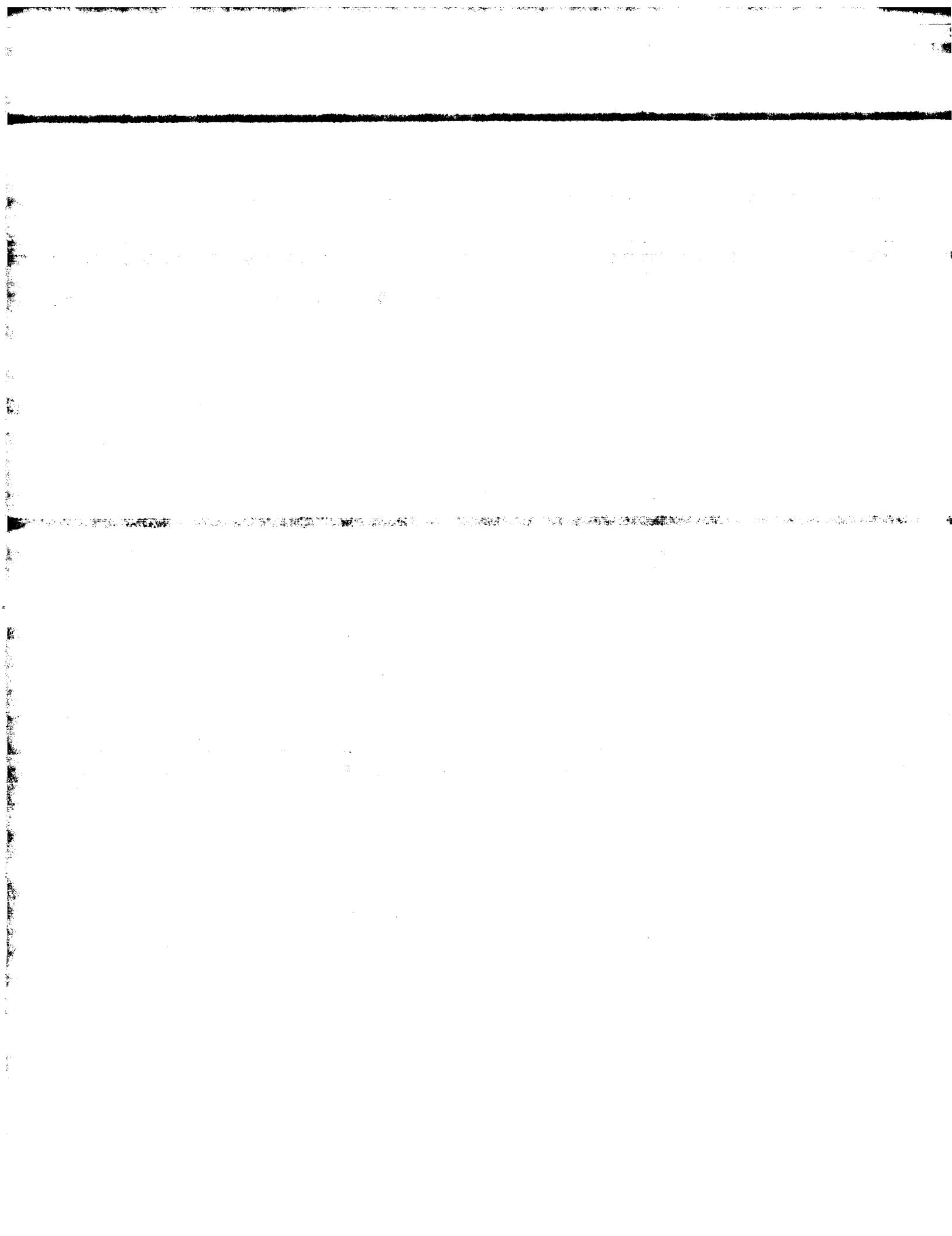




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FIG. 39.





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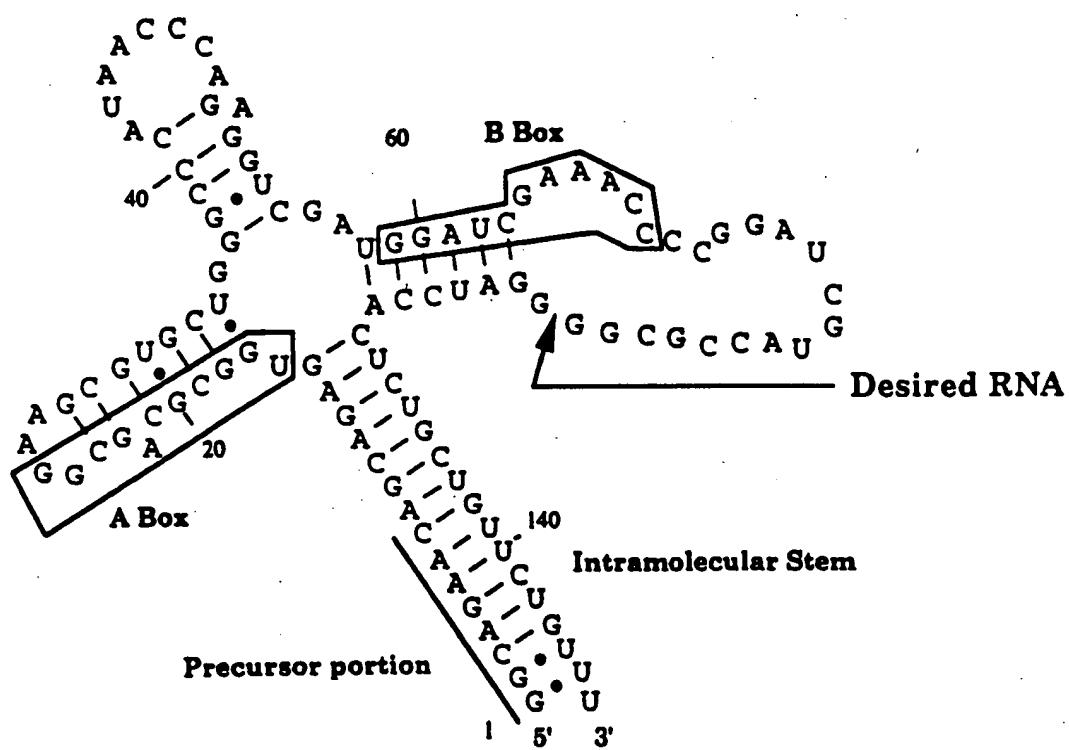


FIG. 40.

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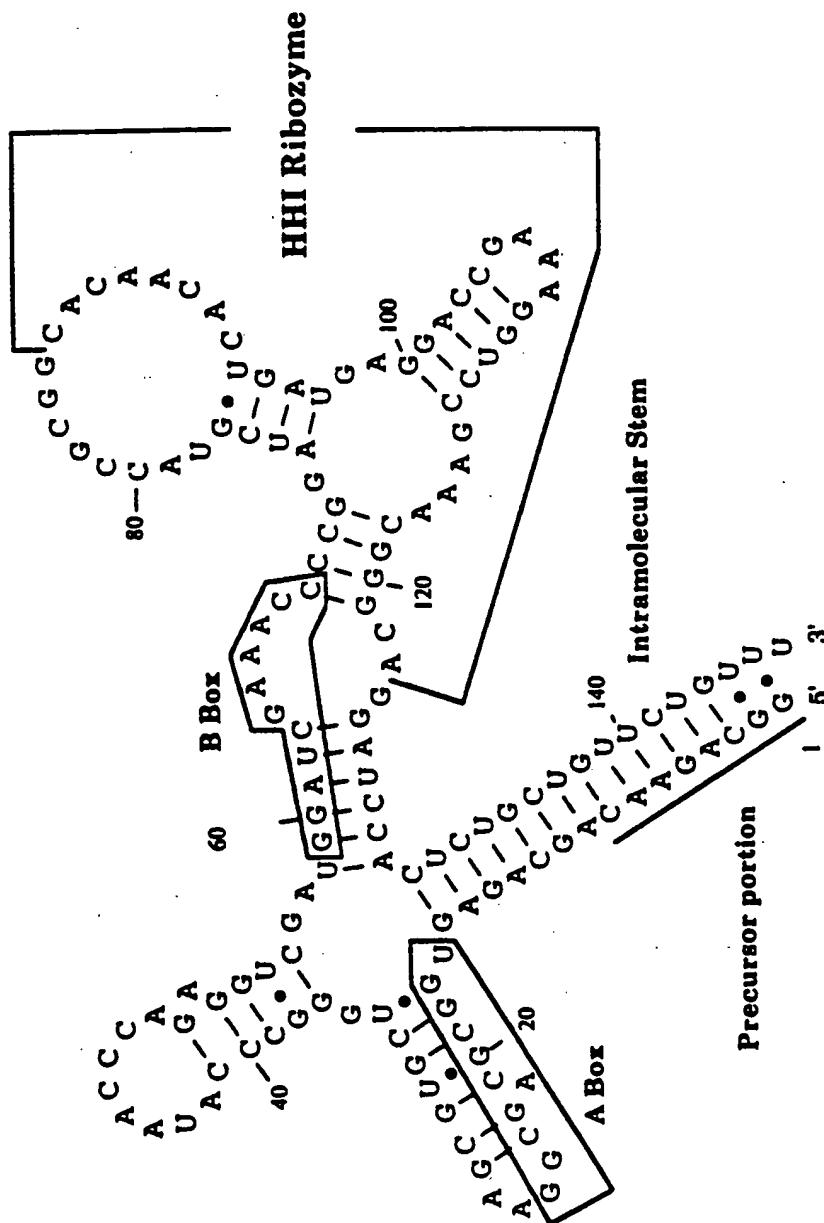


FIG. 41.

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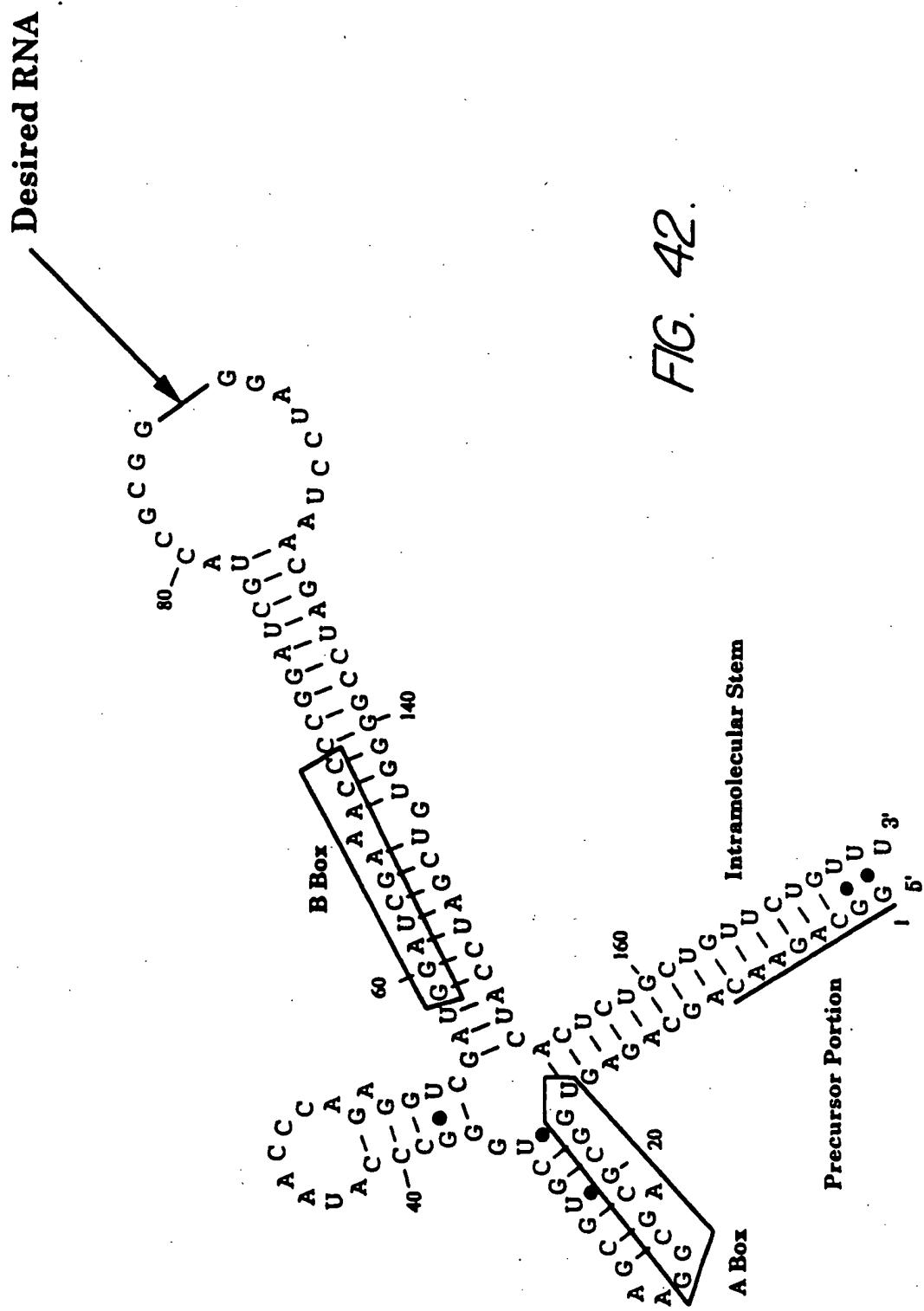
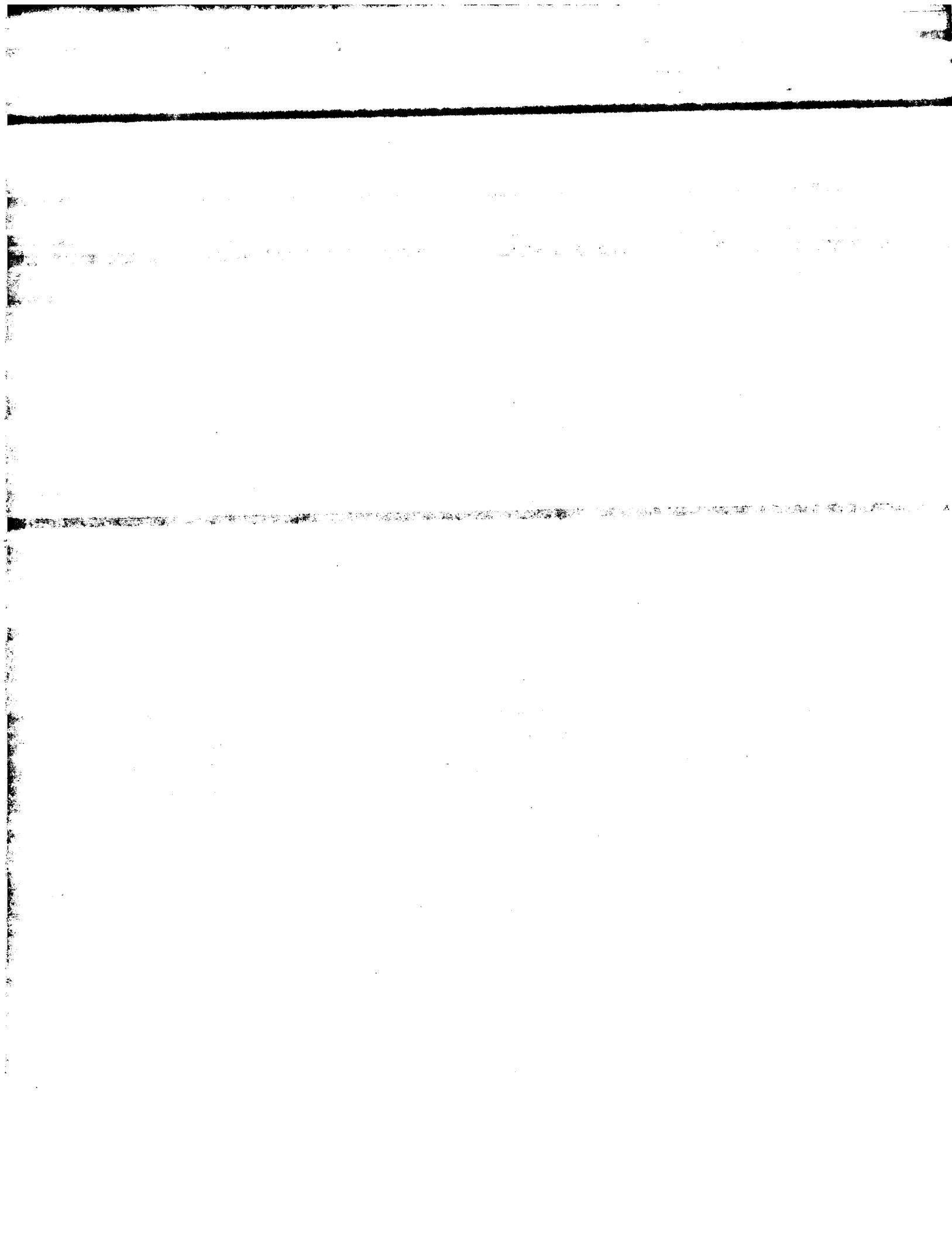
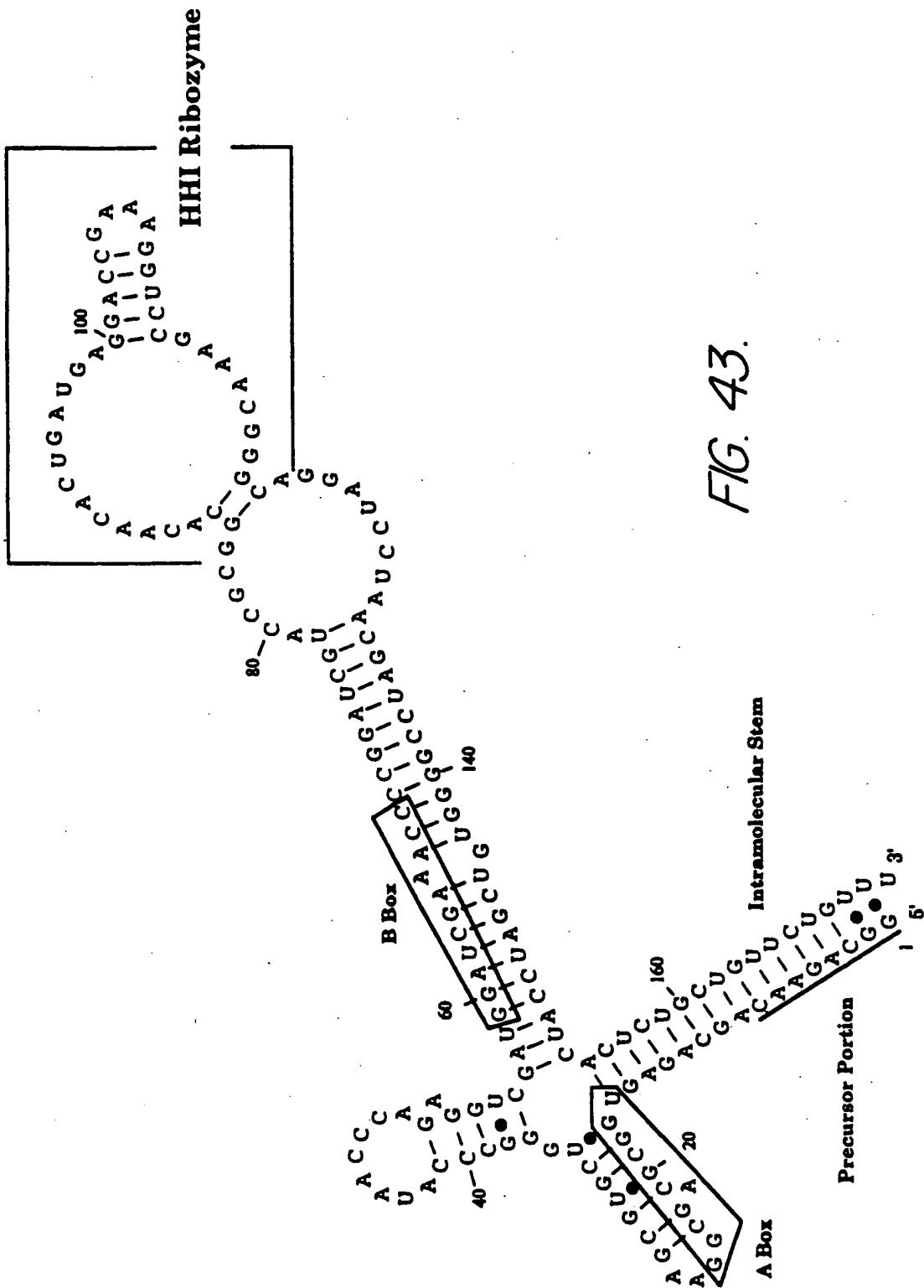
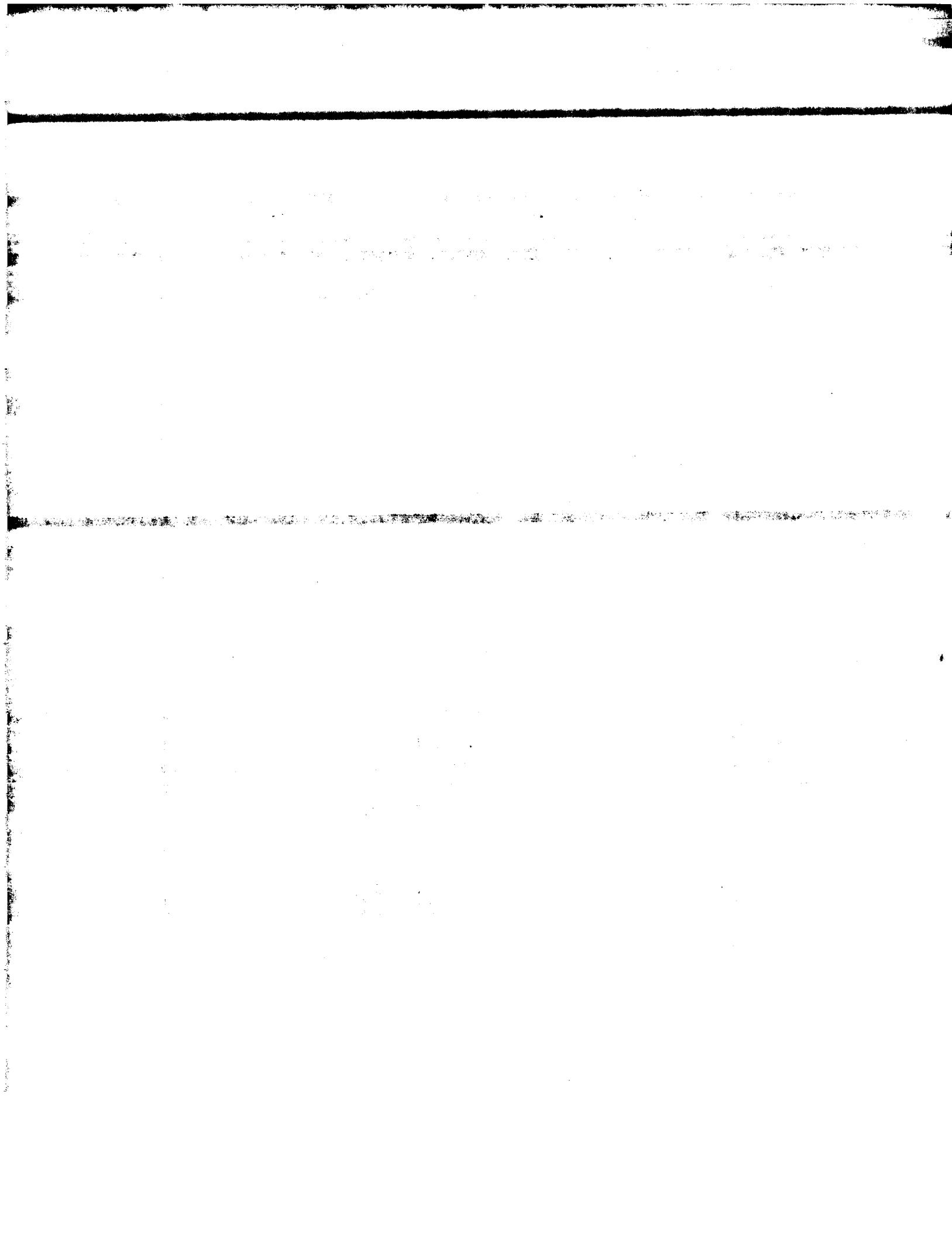


FIG. 42.



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*FIG. 44.***S35 Sequence**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU 100
GUUCUGUUU 109

*FIG. 45.***HHIS35**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGU CUGUUU 146

Underlined bases indicate the HHI ribozyme sequence

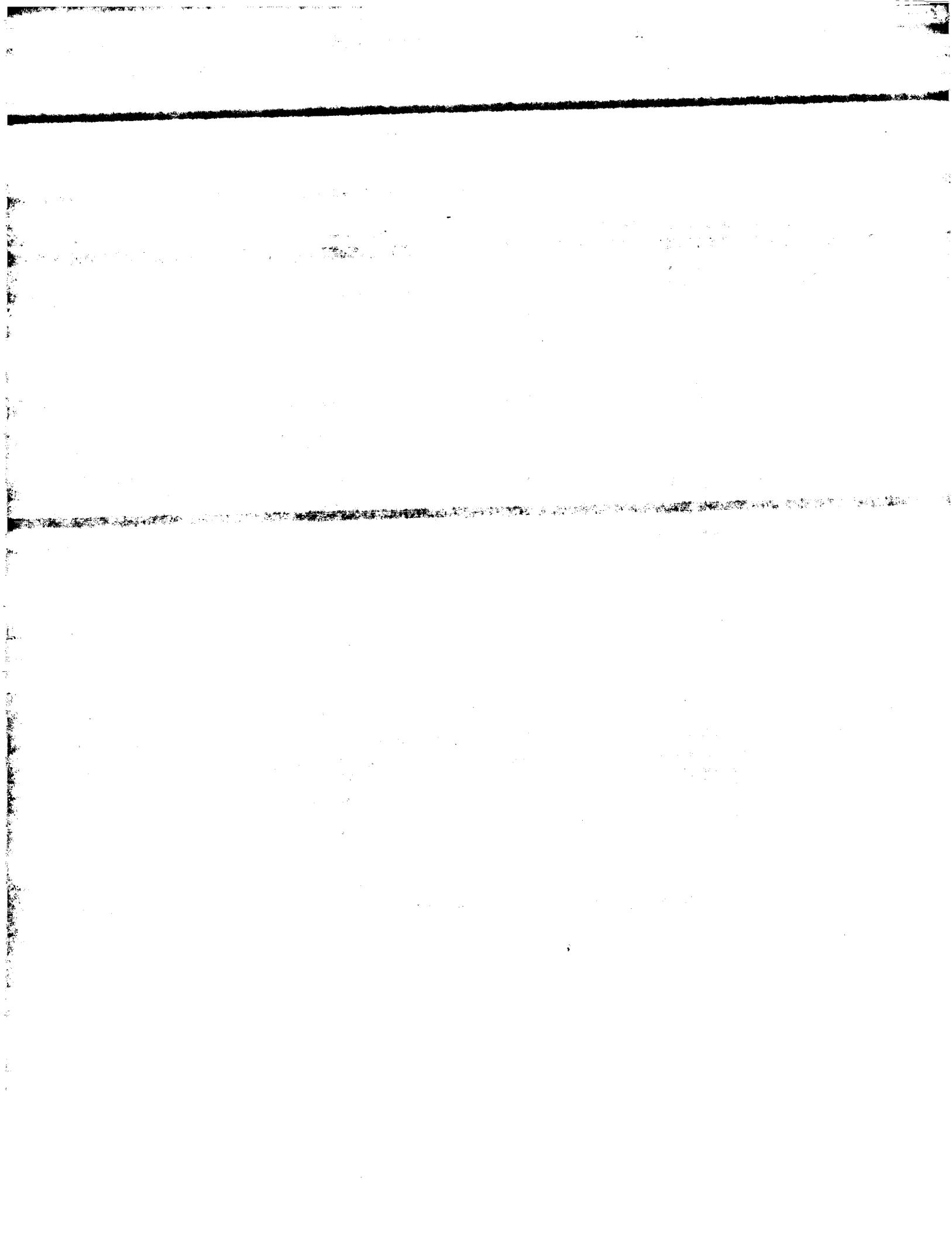
*FIG. 46.***S35 Plus Sequence**

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC 100
GGGGUGUCGA UCCAUCACUC UGCUGUUCUG UU U 133

*FIG. 47.***HHIS35 Plus**

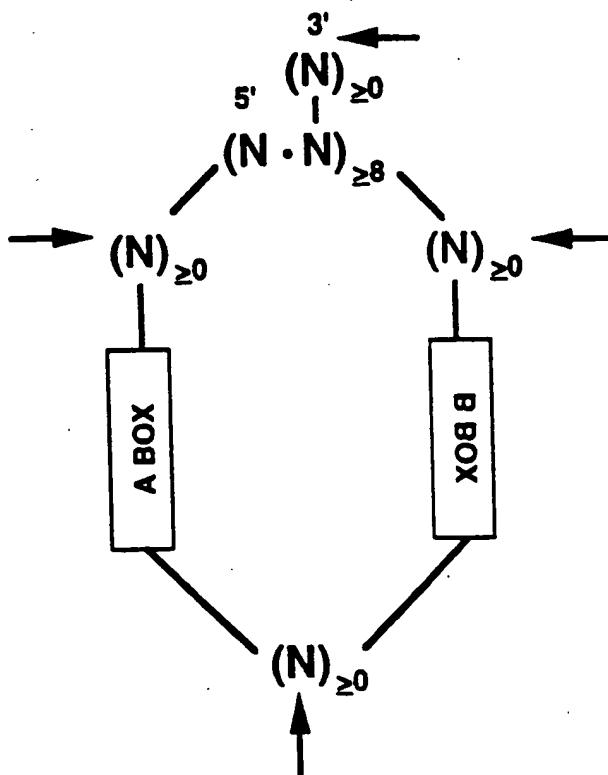
GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC 150
CAUCACUCUG CUGUUCUGUU U 171

Underlined bases indicate the HHI ribozyme sequence
 SUBSTITUTE SHEET (RULE 26)



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FIG. 48.

**A BOX = URGCNNAGYGG****B BOX = GGUUCGANUCC**

This is based on Geiduschek & Tocchini-Valentini,
(1988) *Annu. Review Biochem.* 57, 873-914. However
this consensus sequence is not meant to be limiting

N = A, U, G, or C**R = Purine****Y = Pyrimidine**

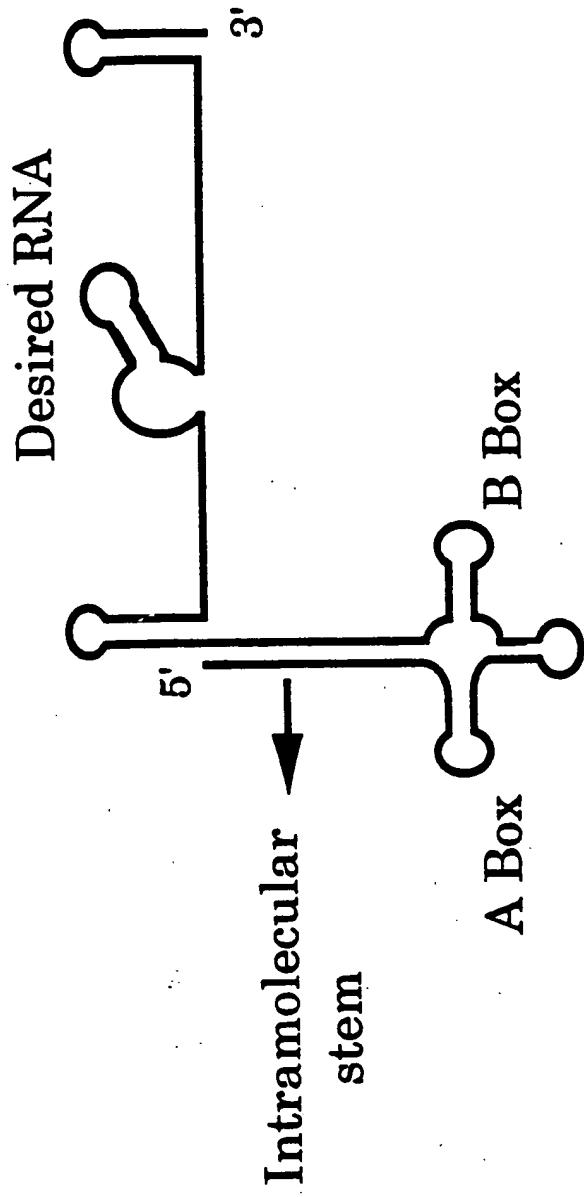
• = Indicates base-pairing

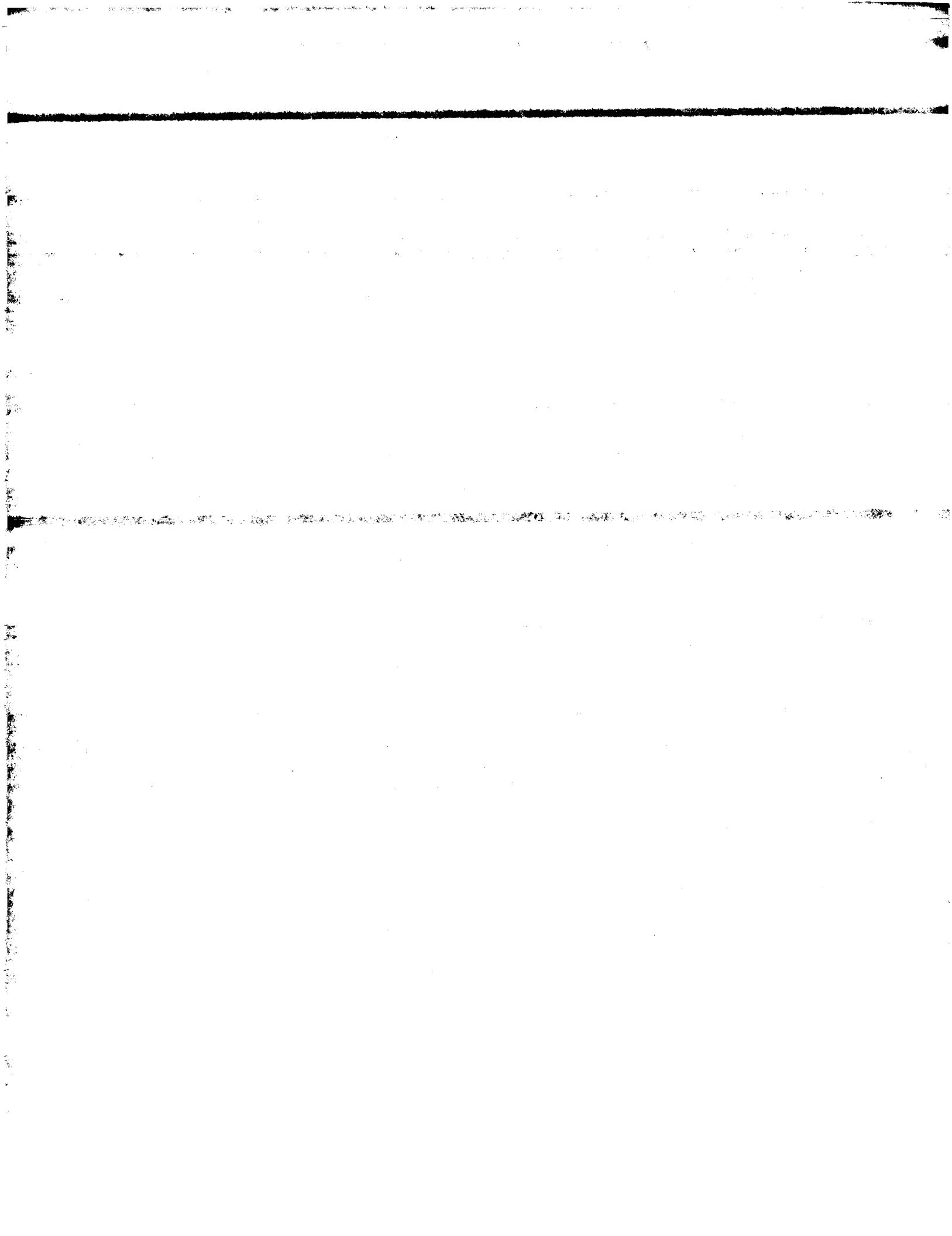
— = Indicates covalent linkage

→ = Indicates sites at which desired
RNAs can be cloned

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FIG. 49.





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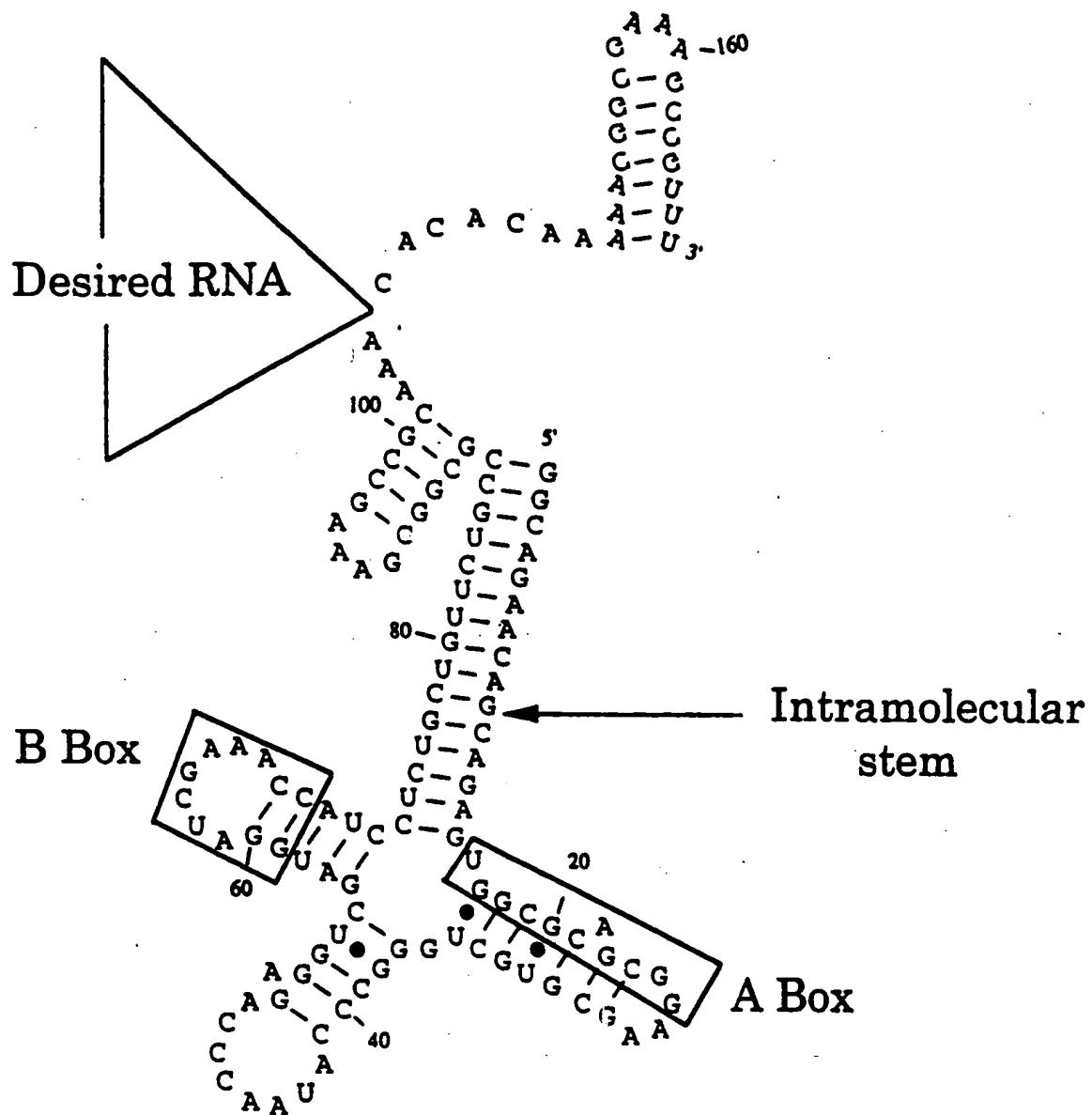
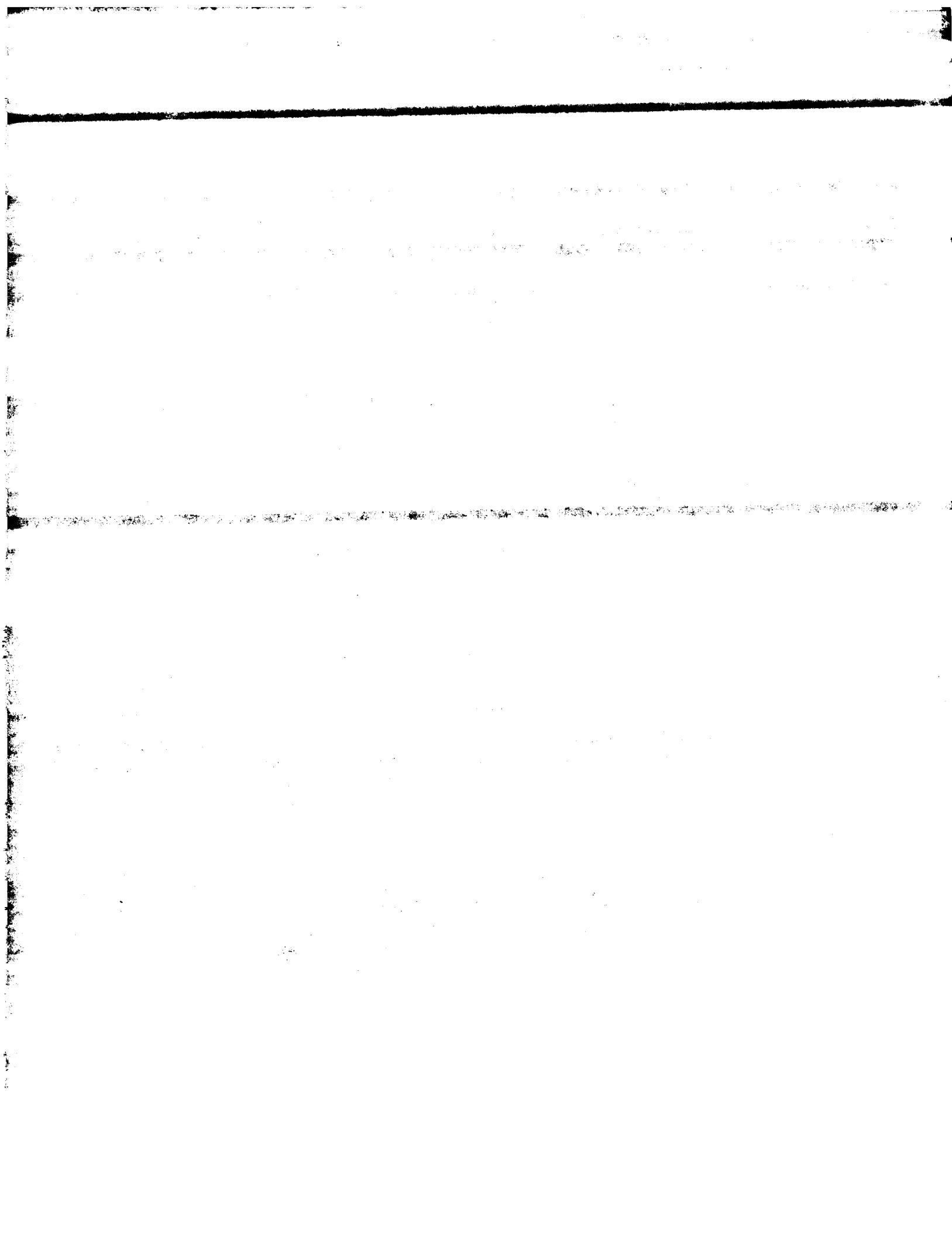


FIG. 50.



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HHI Ribozyme

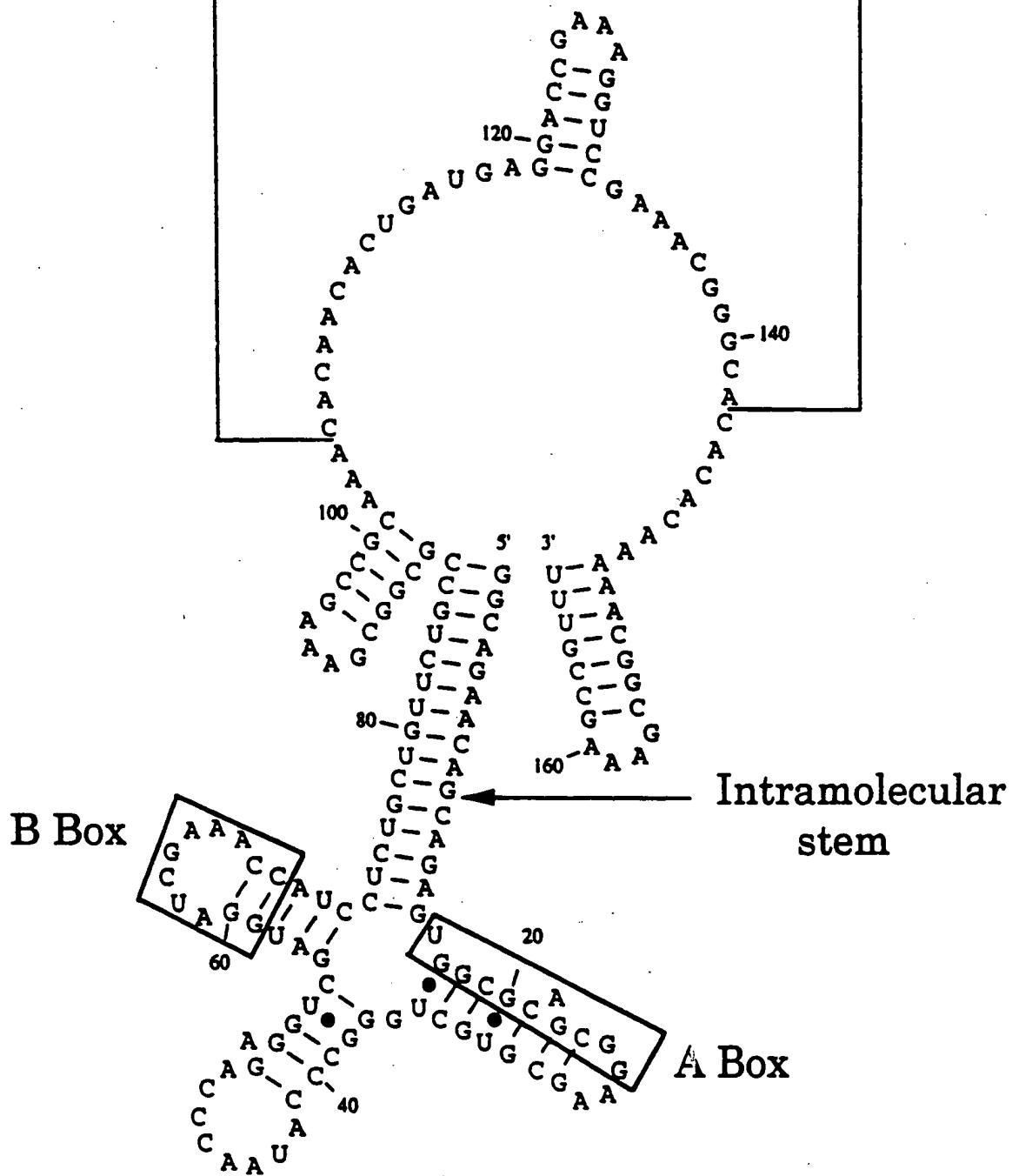


FIG. 51.

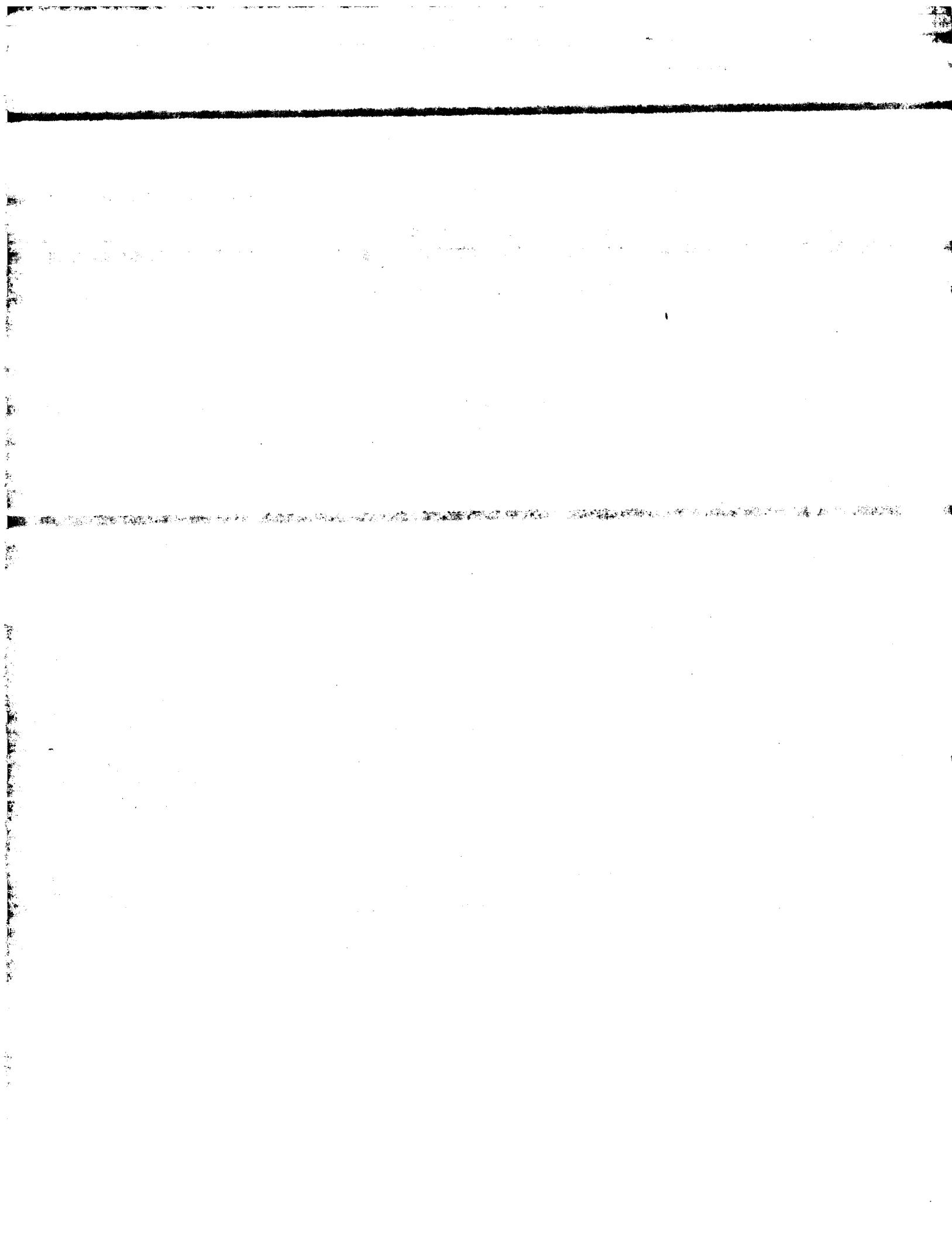


FIG. 52a.

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A: TRZ-A

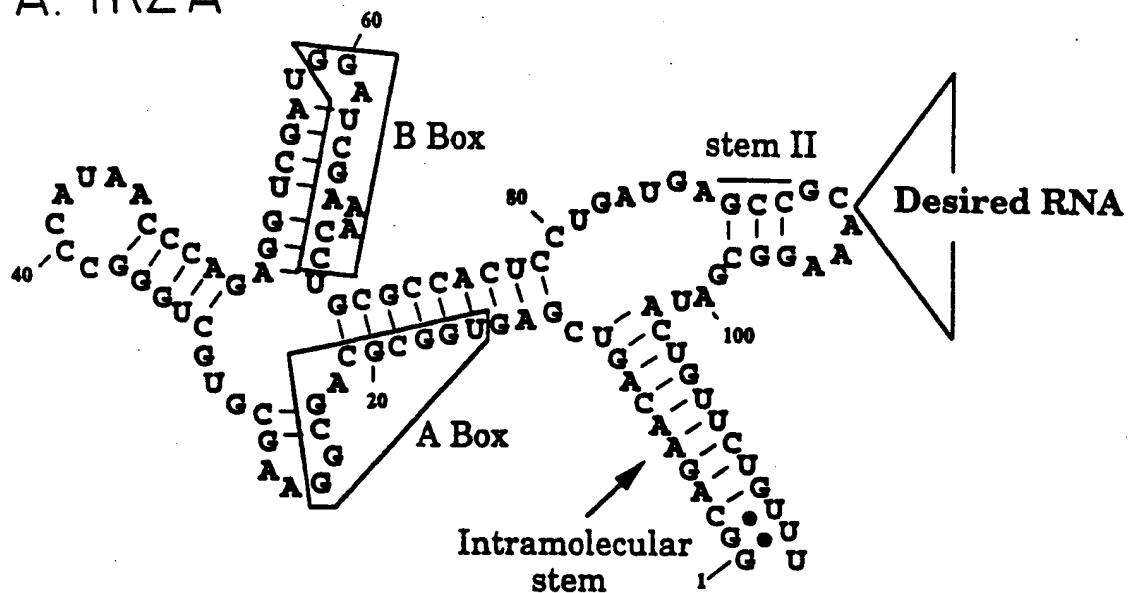
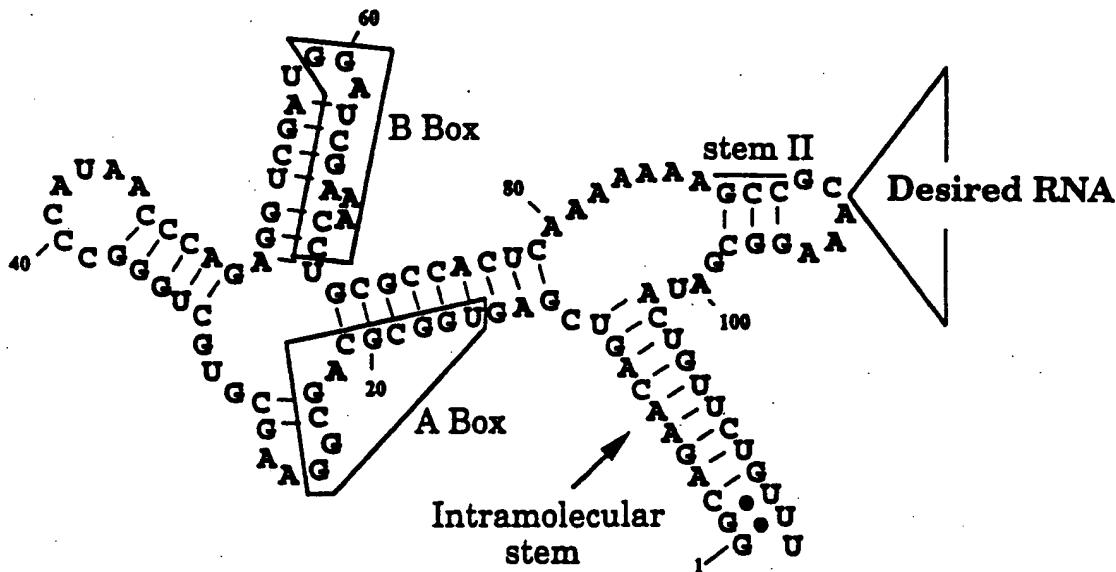
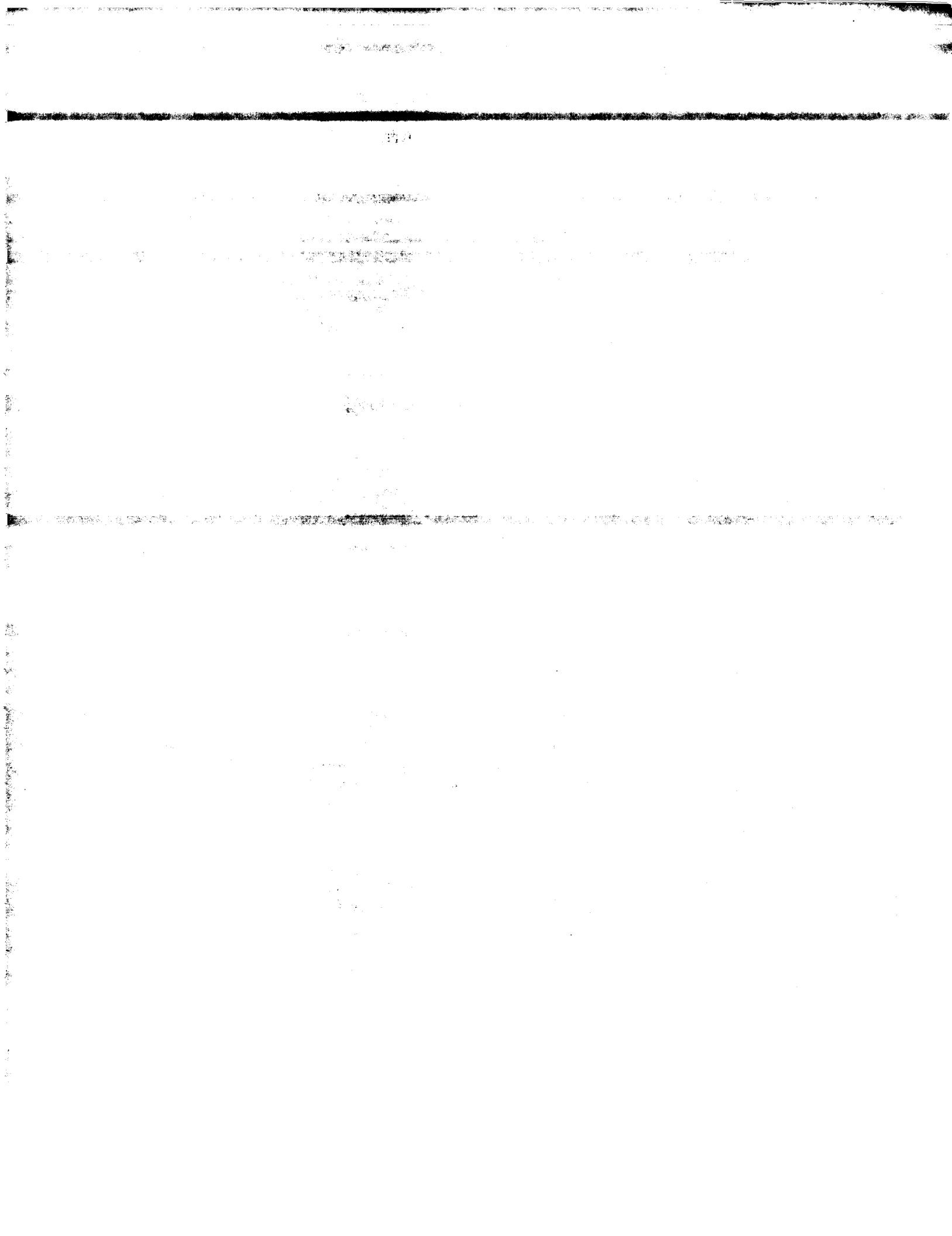


FIG. 52b.

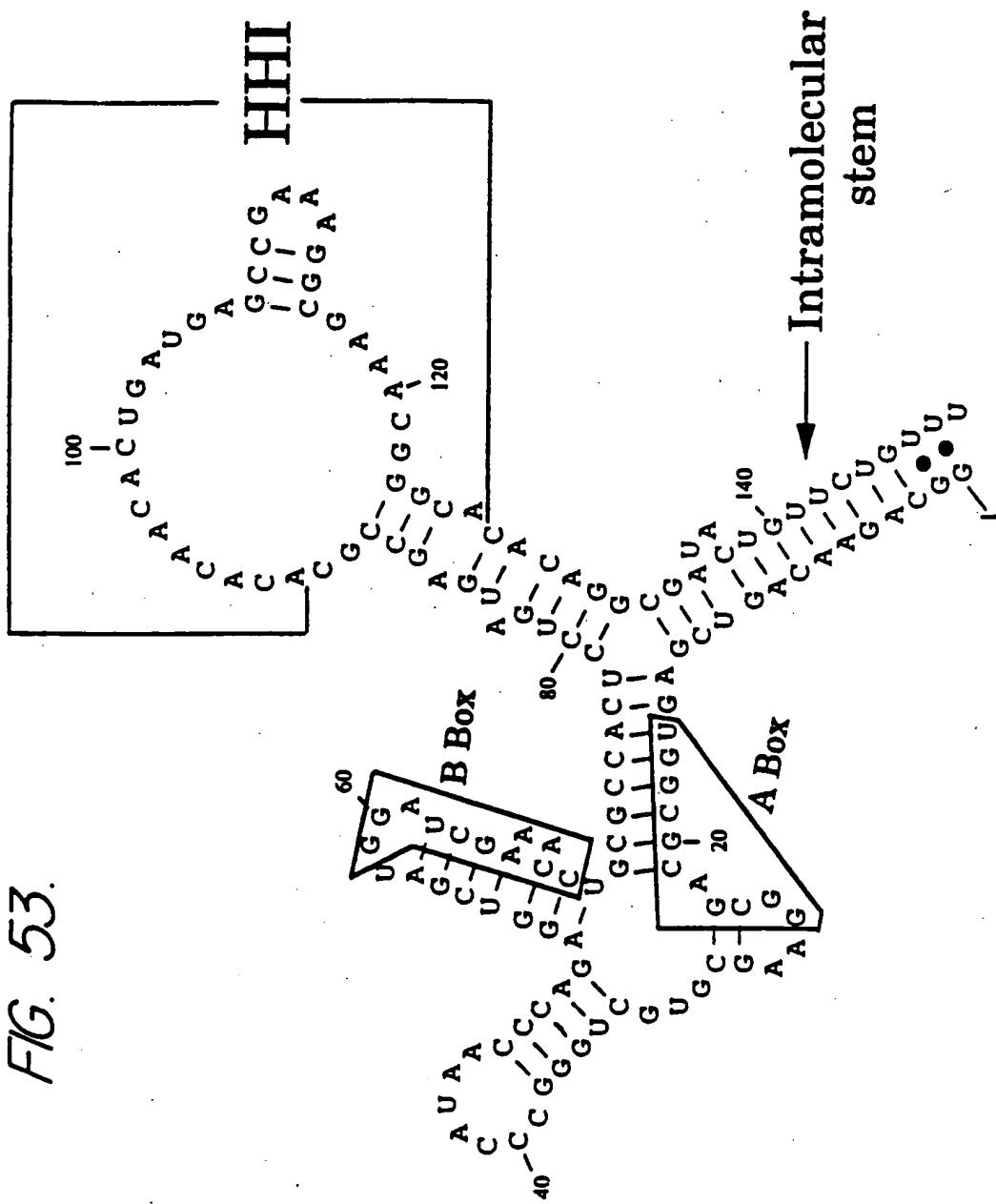
B: TRZ-B

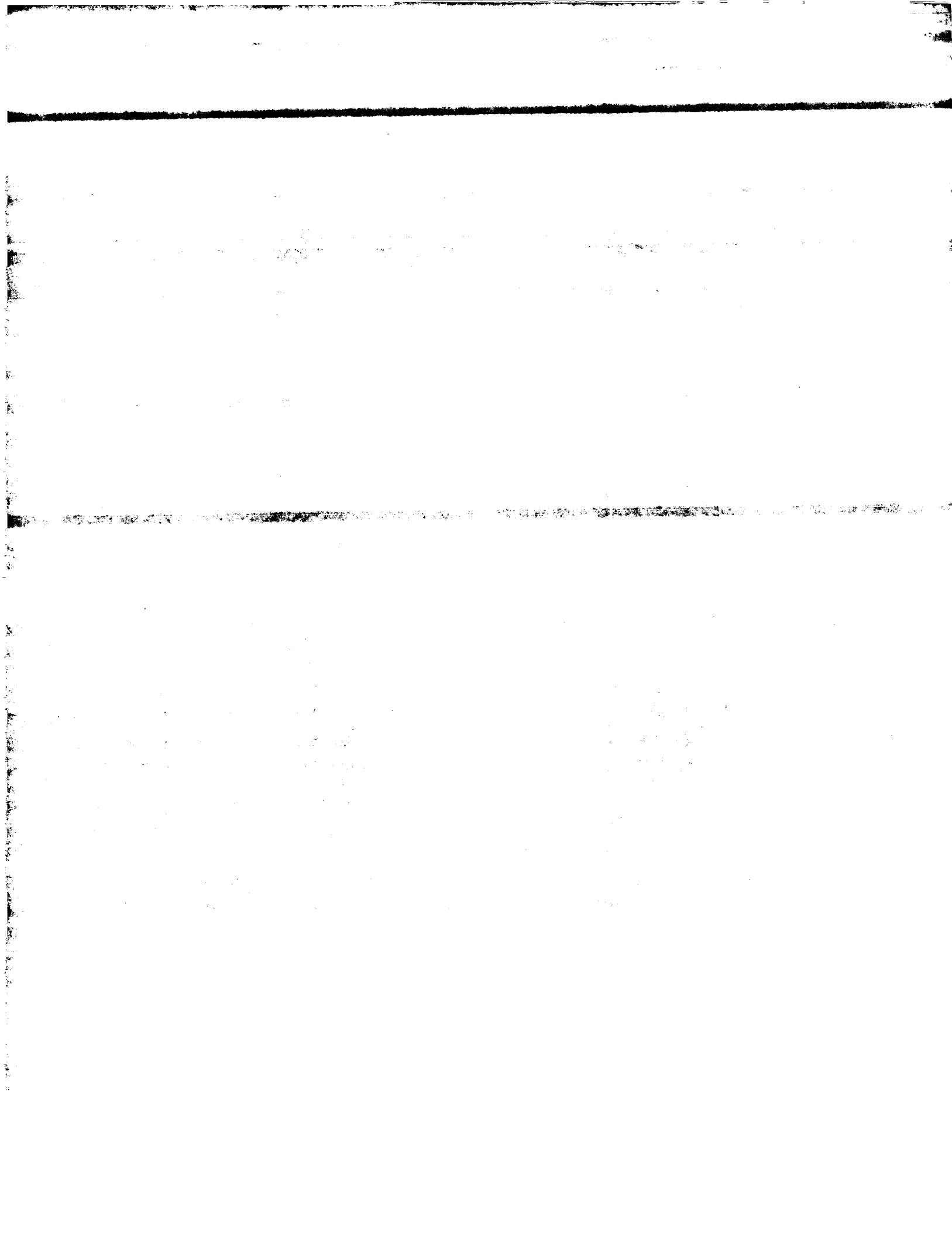




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FIG. 53.





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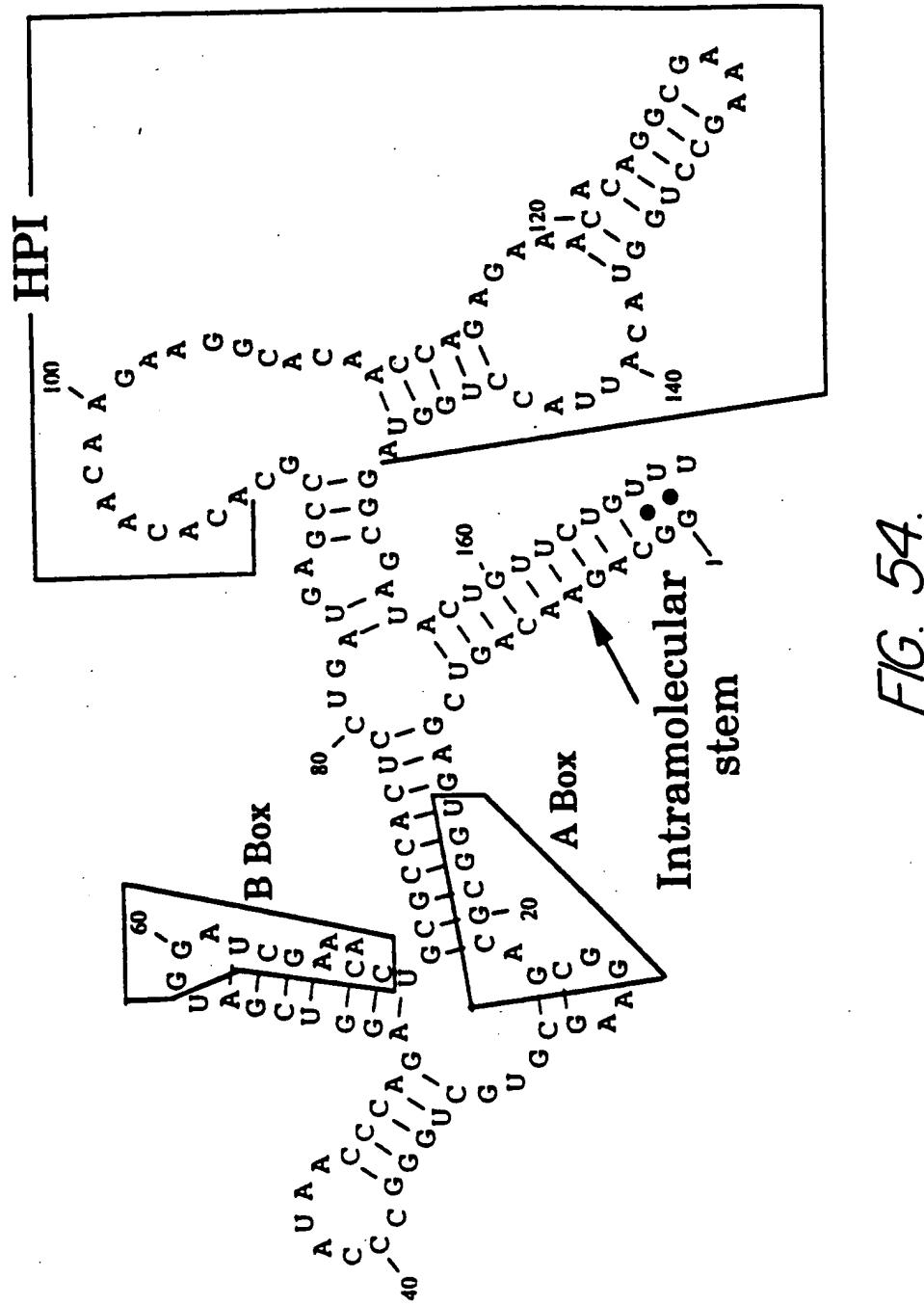
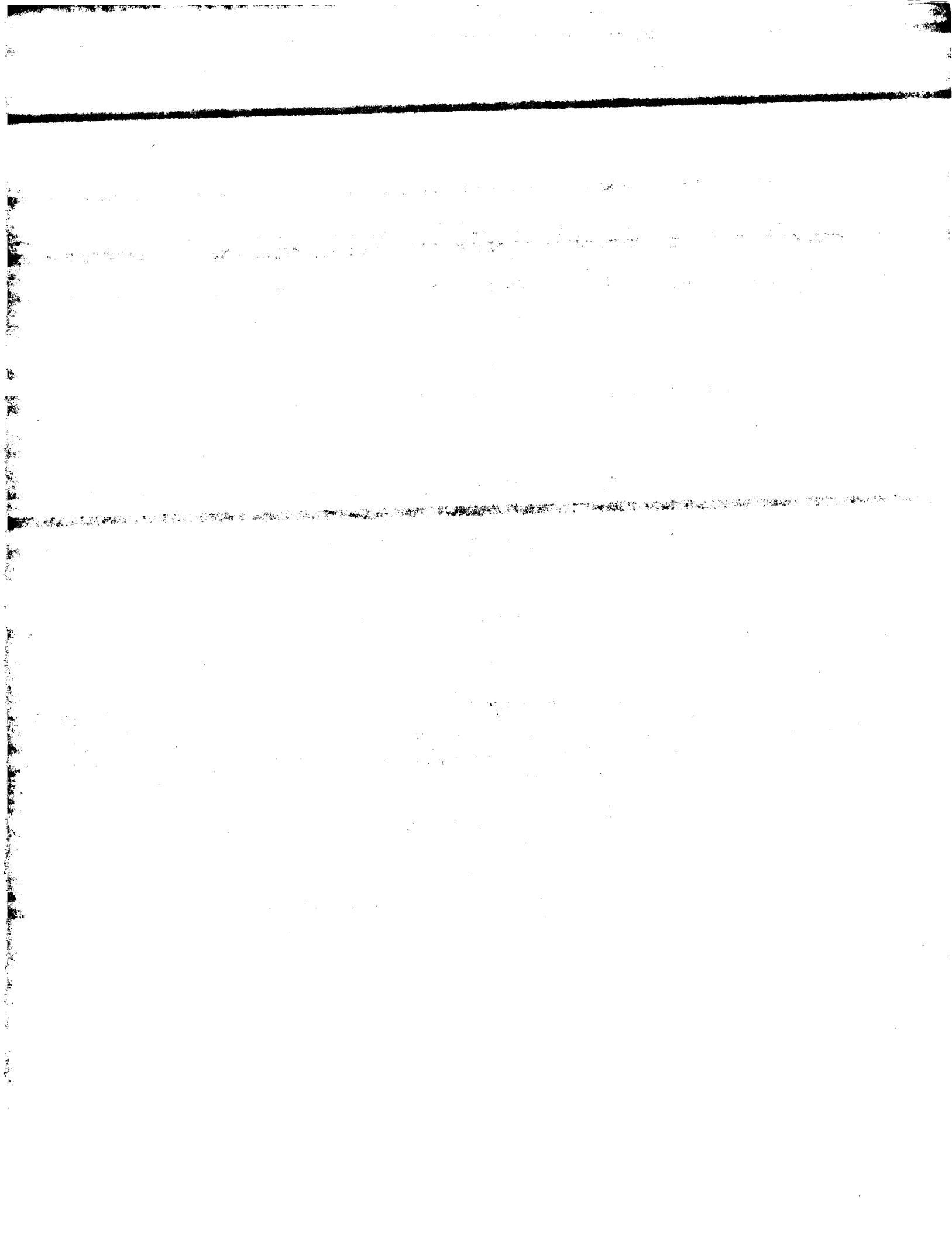


FIG. 54.



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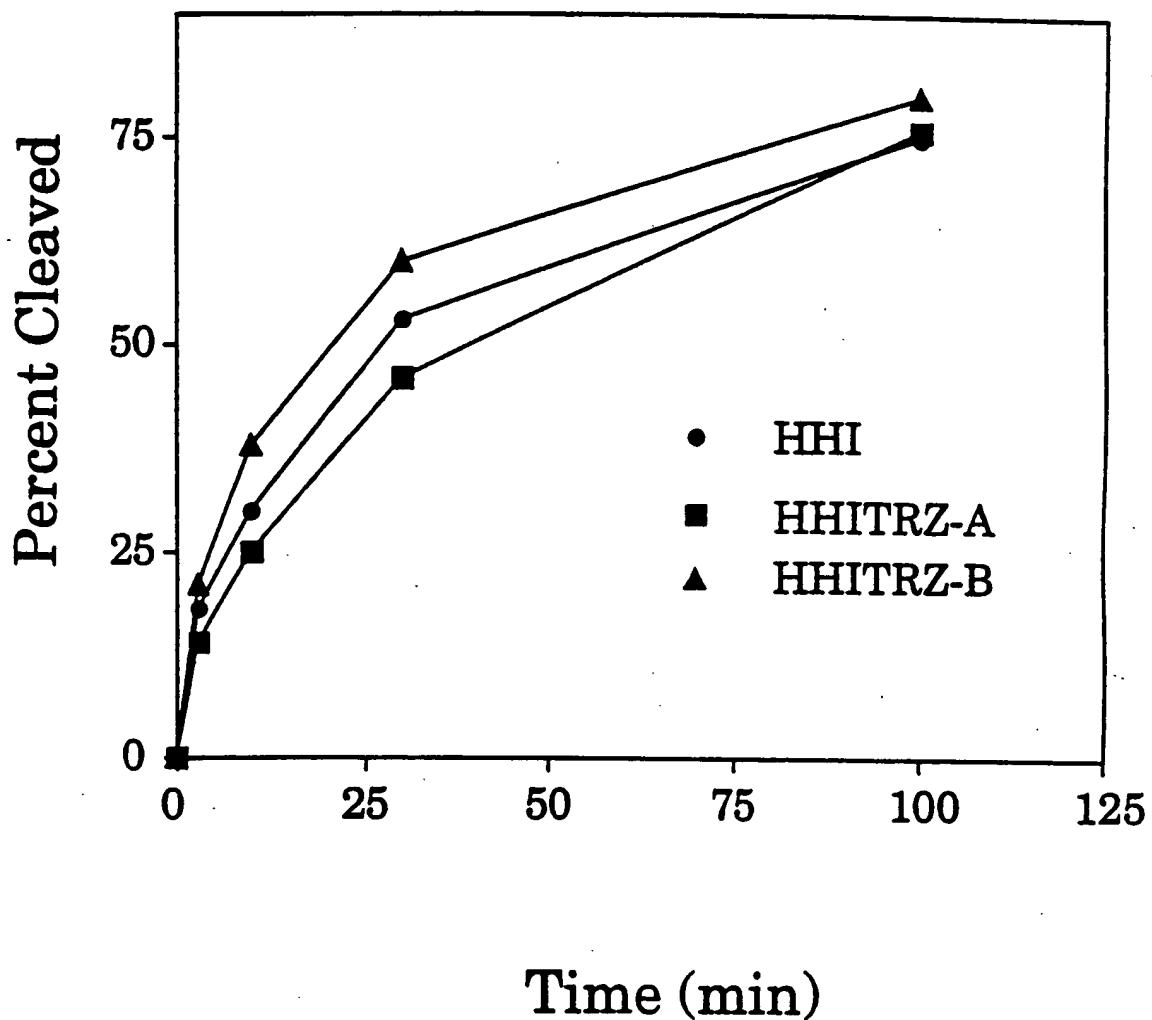
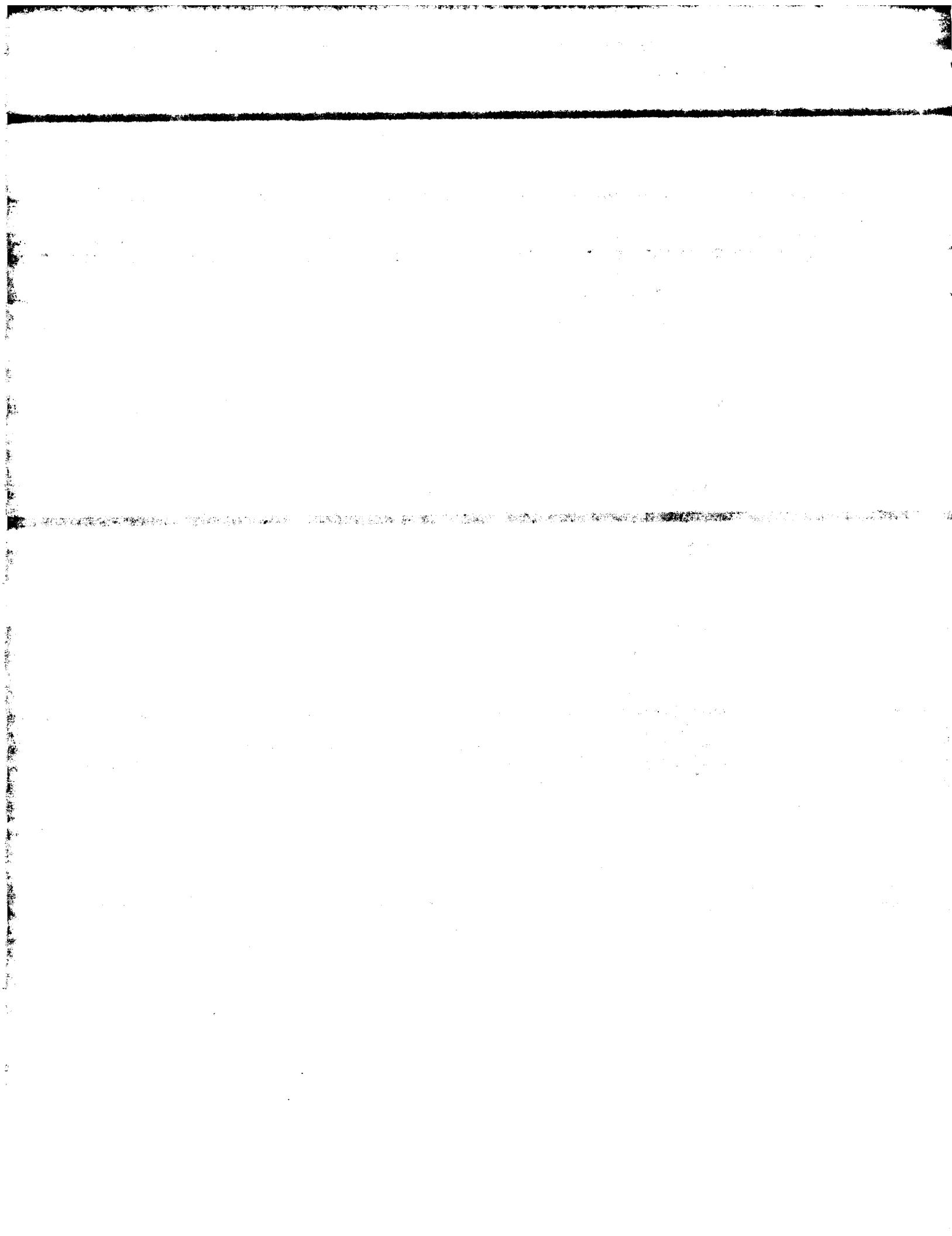


FIG. 55.



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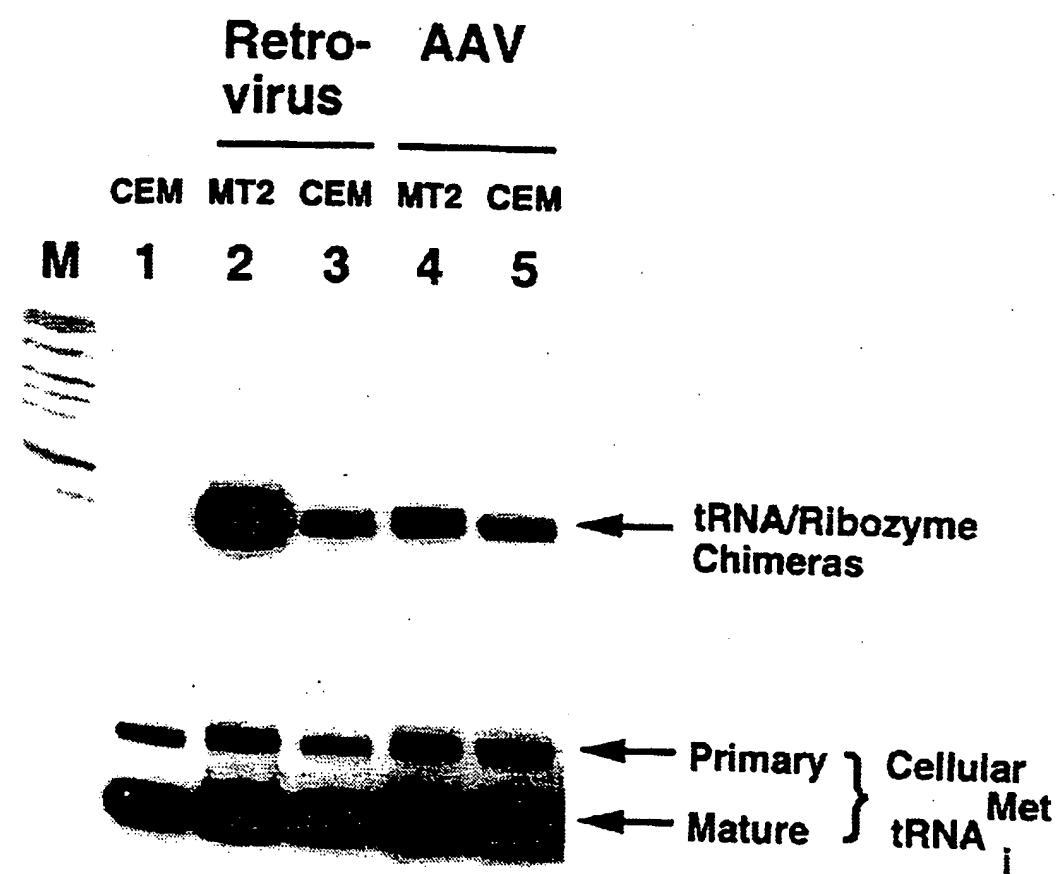
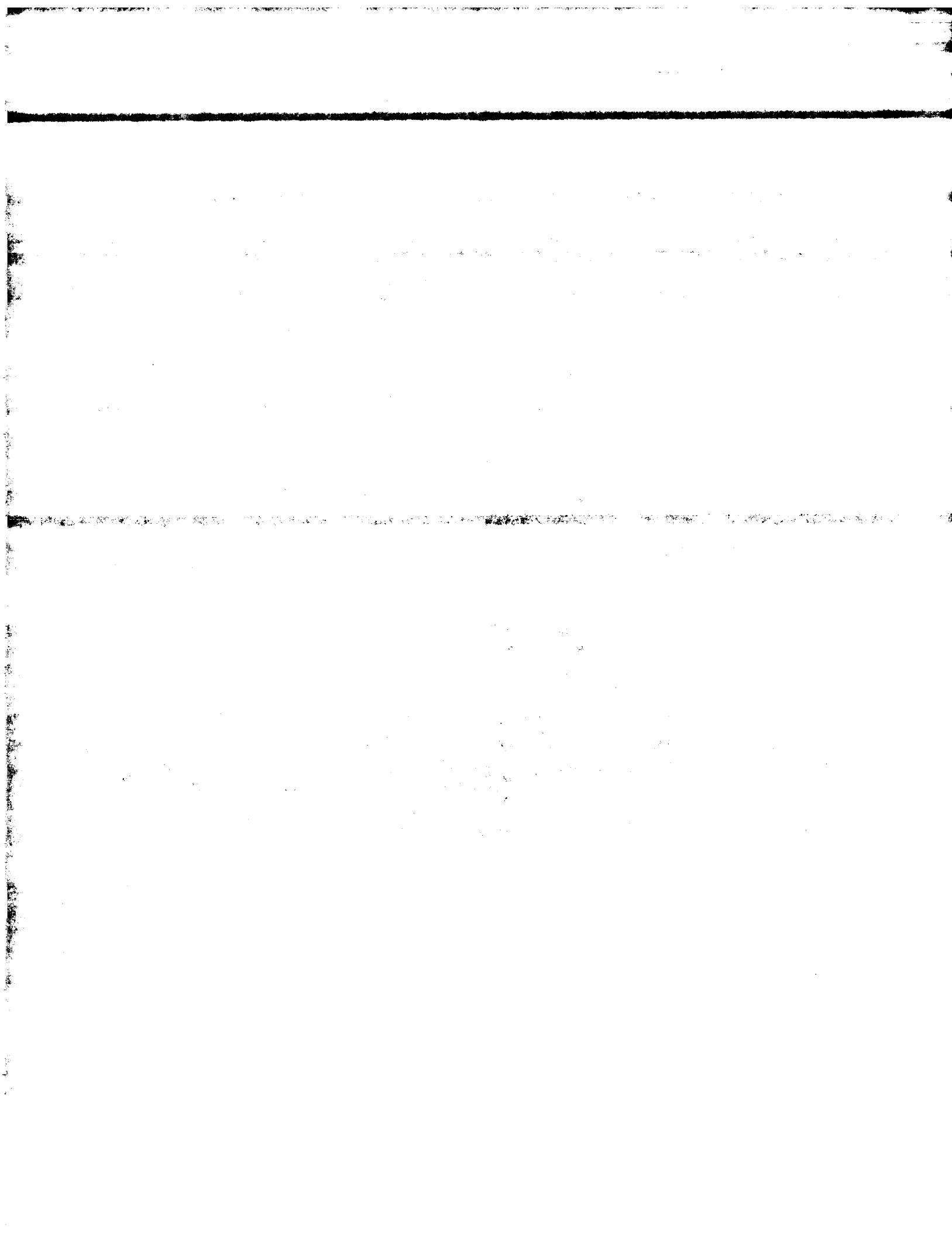


FIG. 56.



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FIG. 57a.

AAV Vector

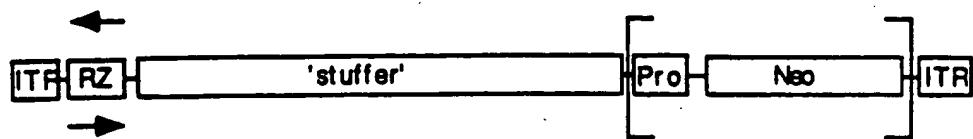
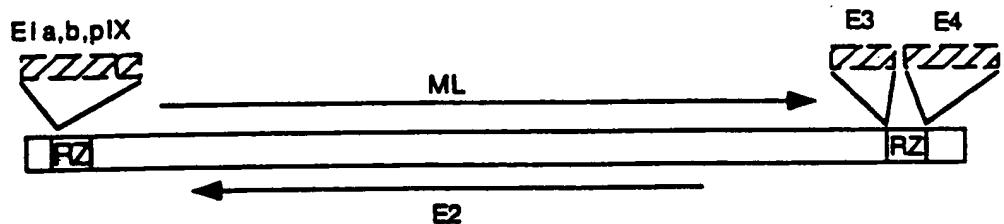
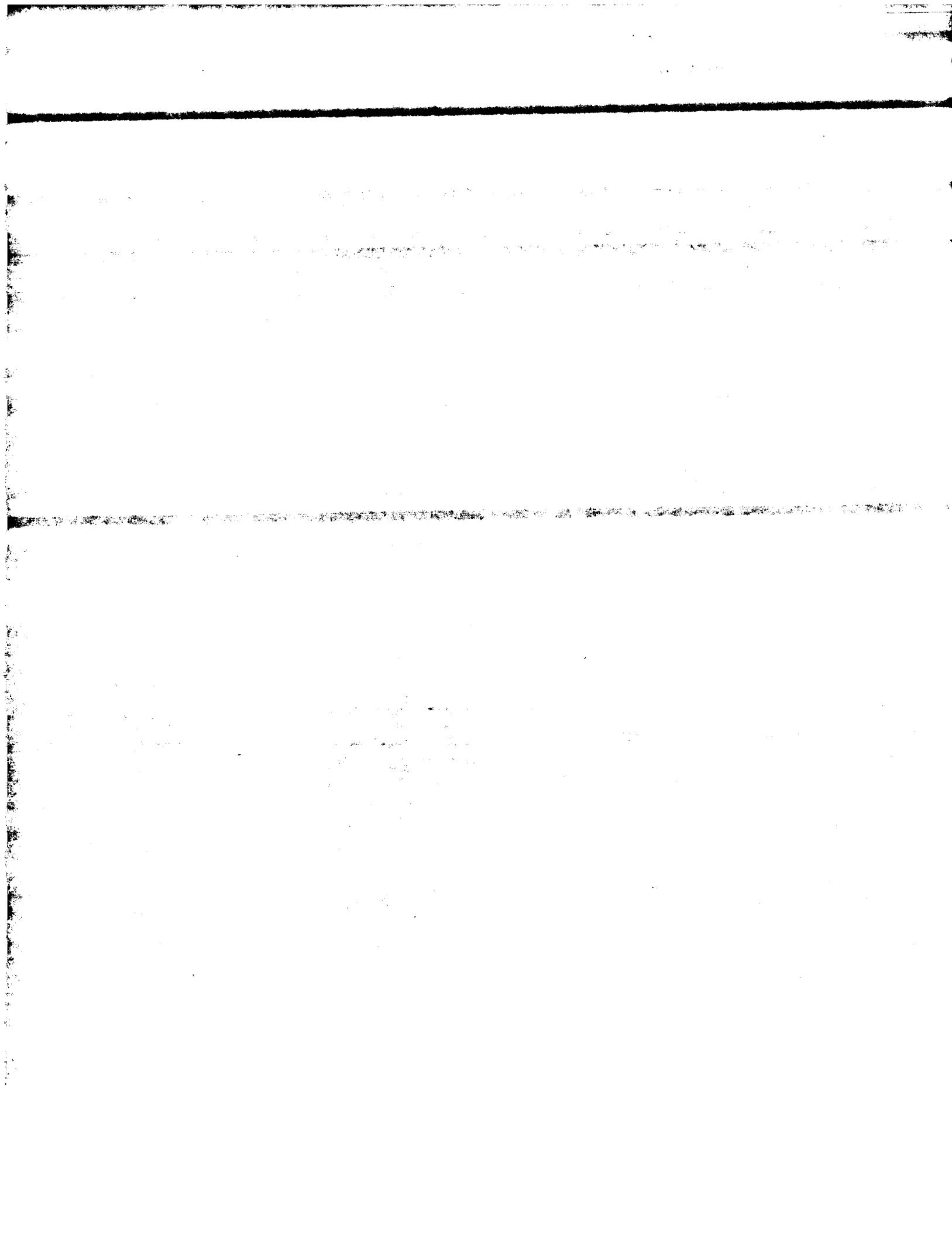


FIG. 57b.

Adenovirus Vector





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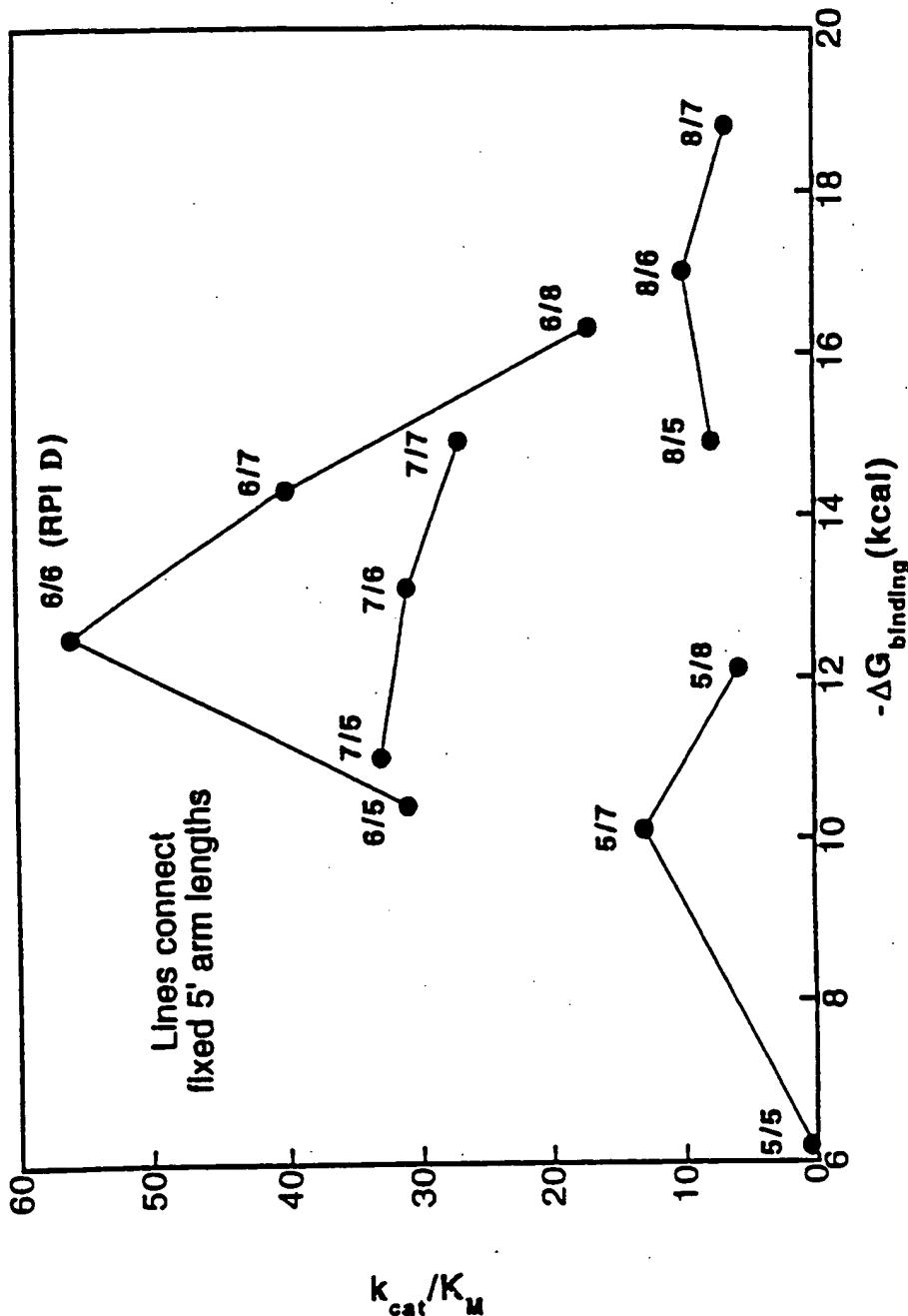
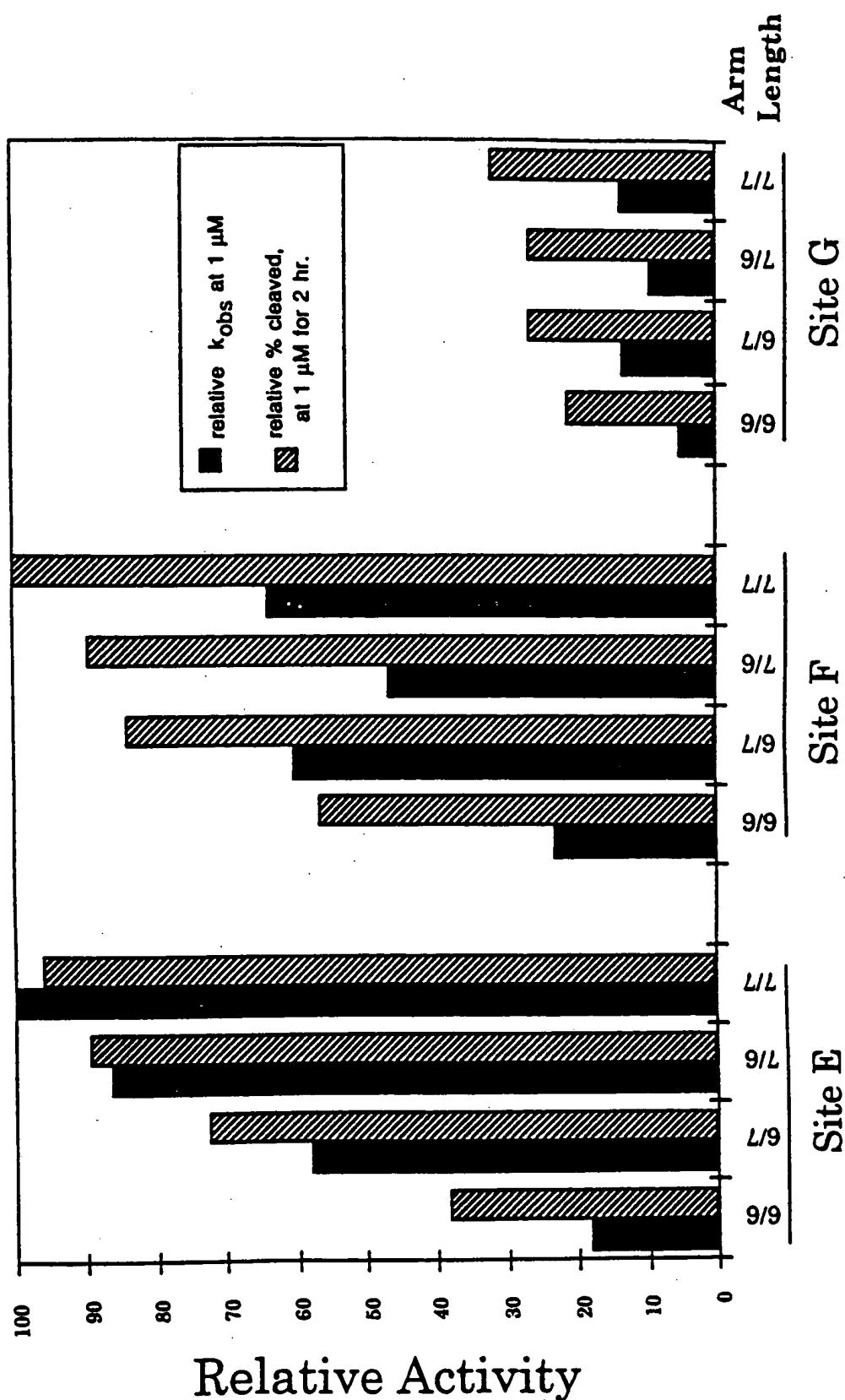


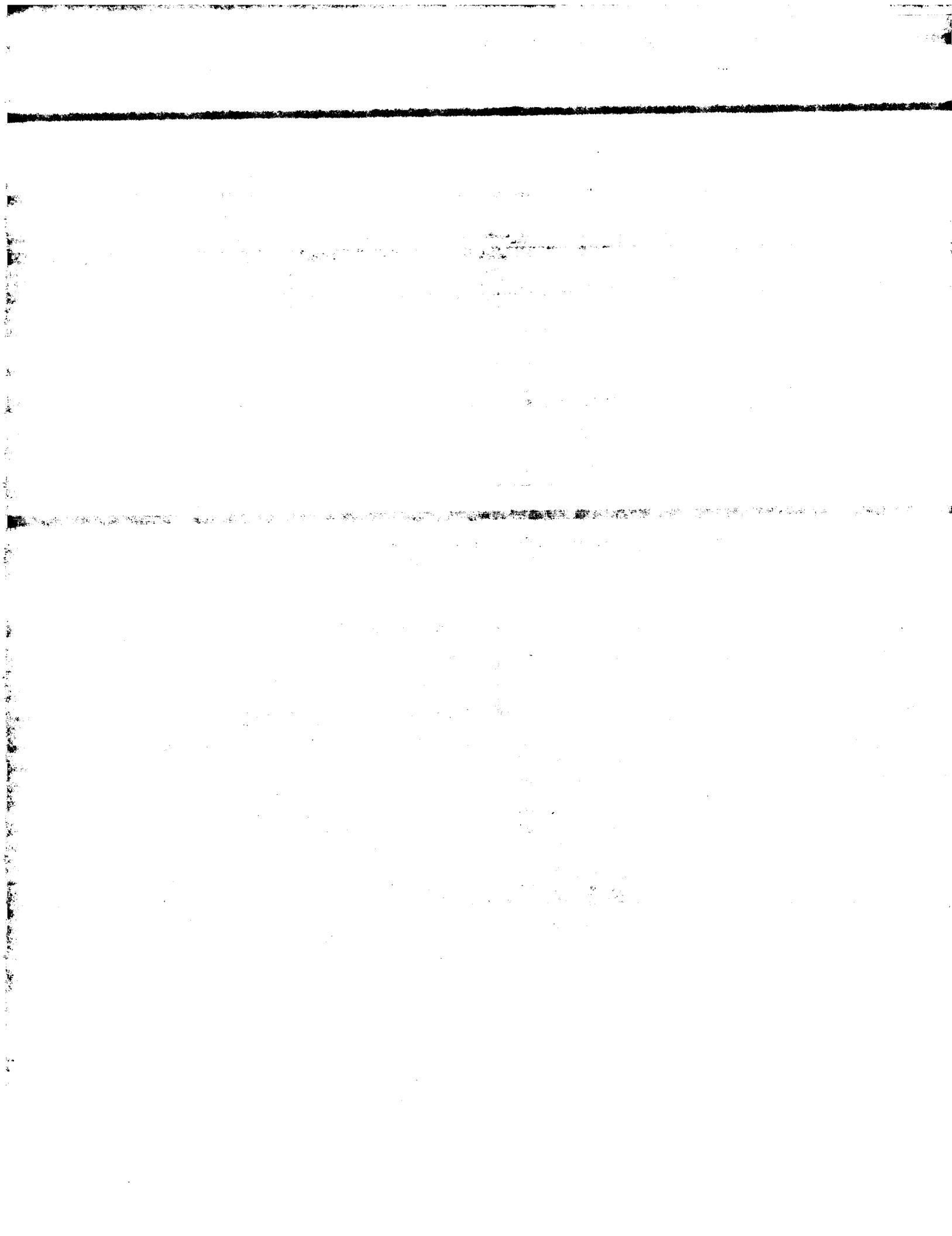
FIG. 58.

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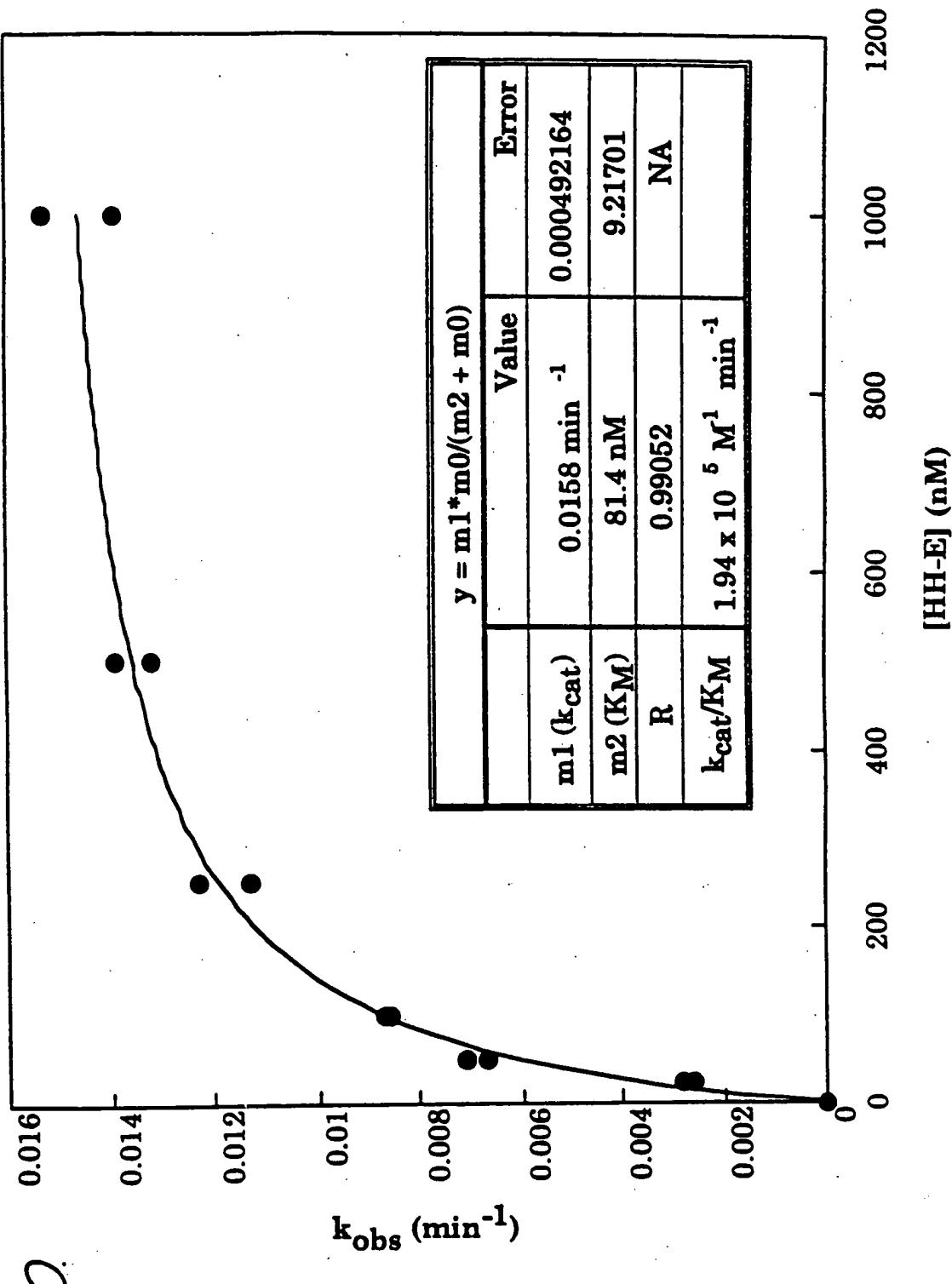


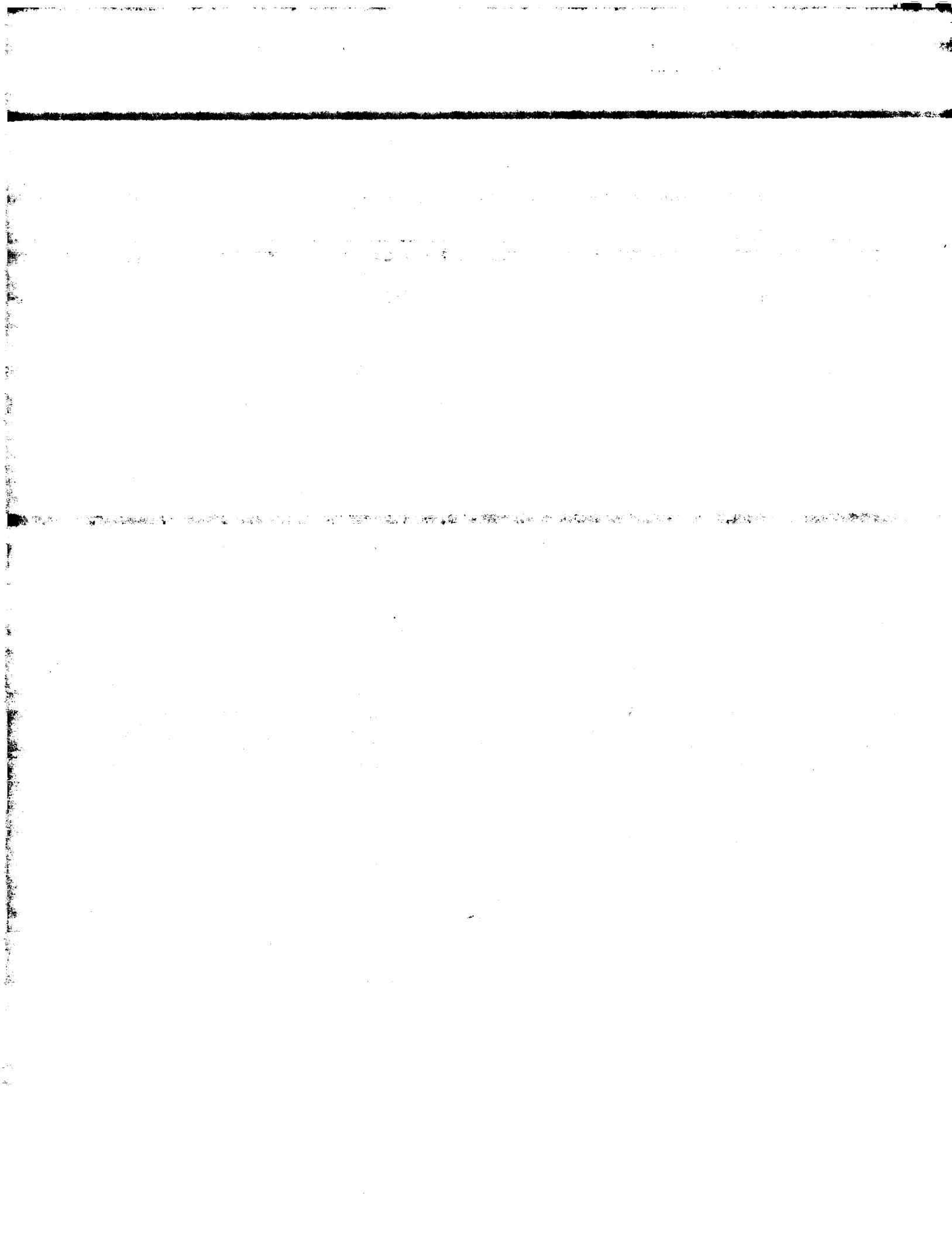
Ribozyme

FIG. 59.

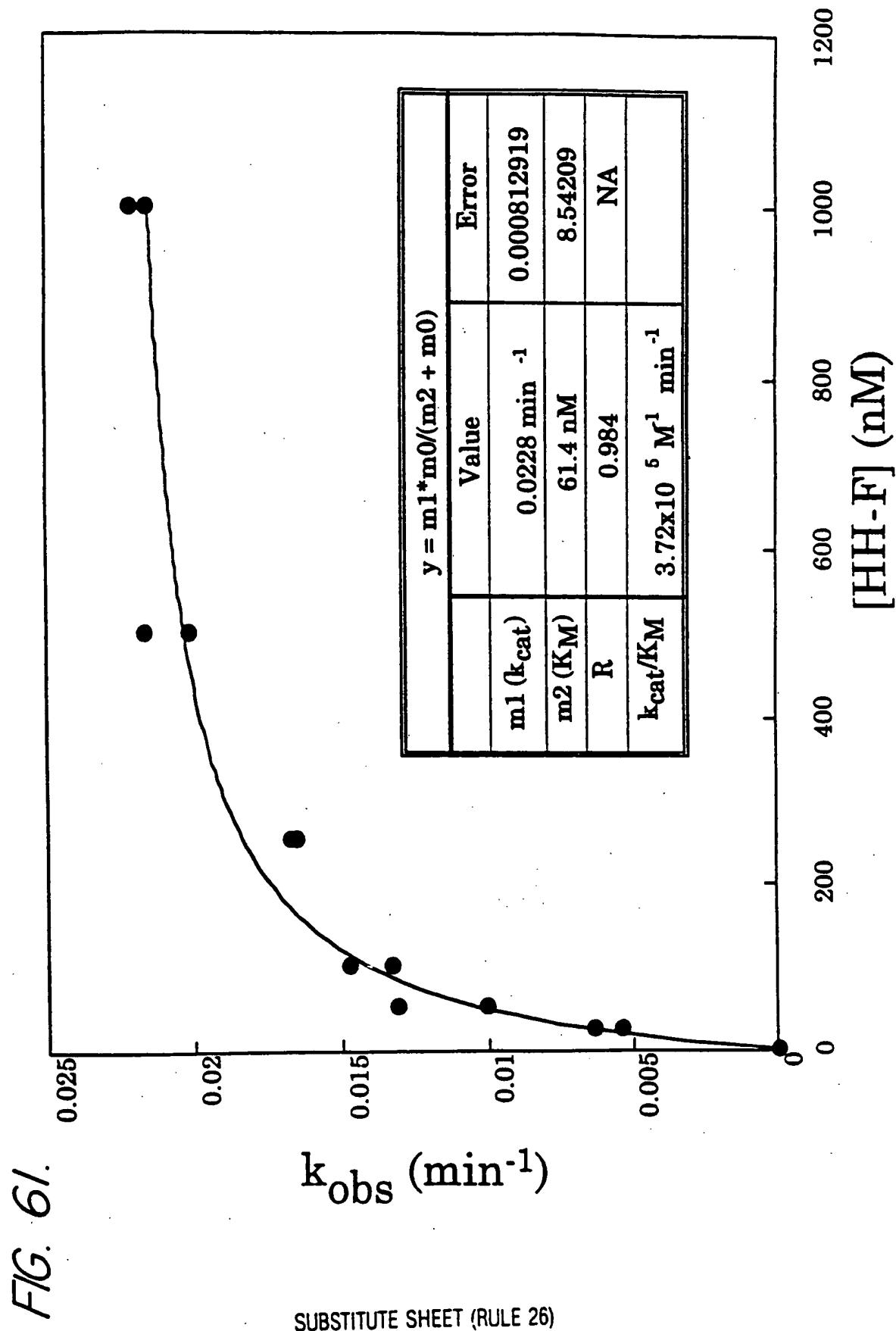


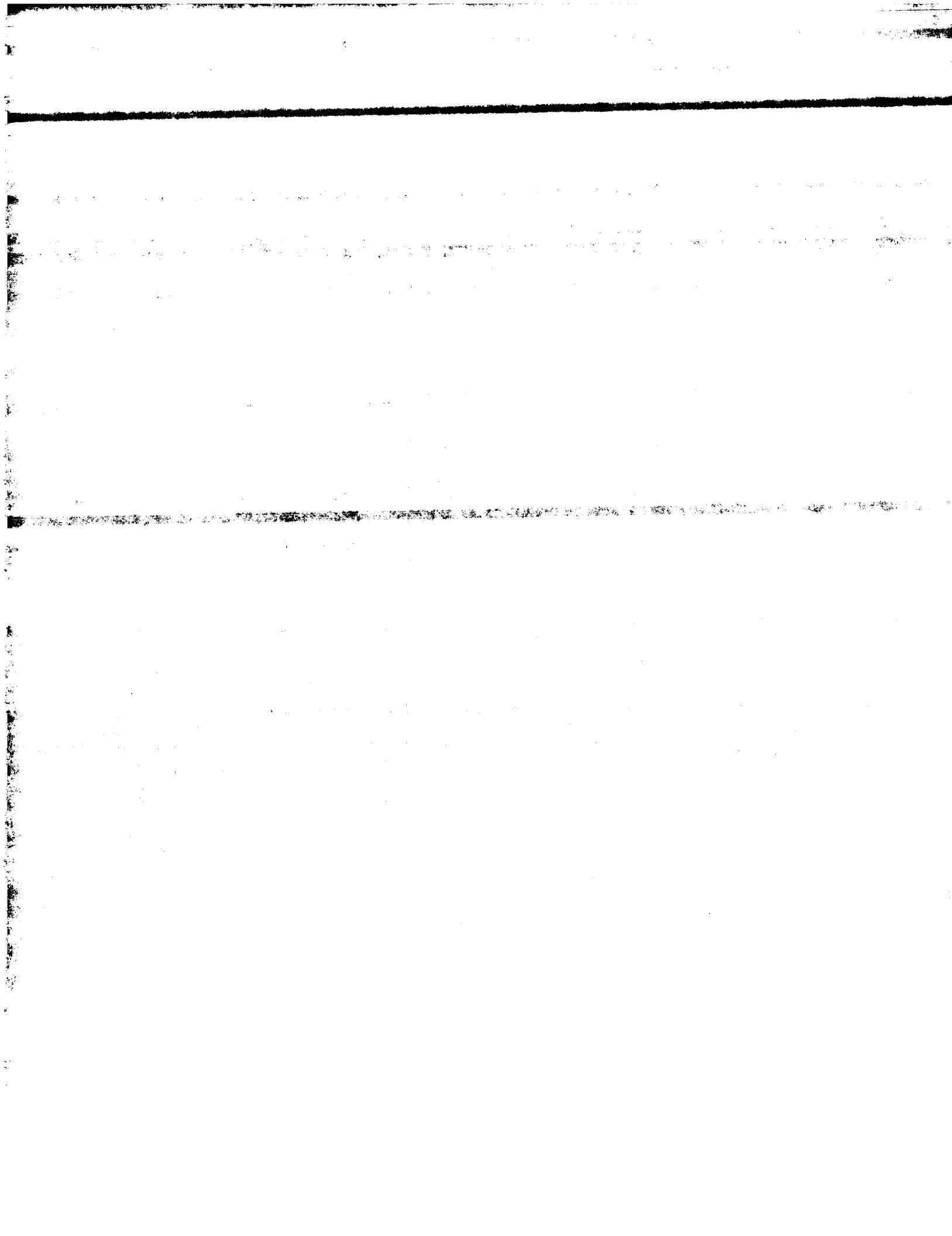
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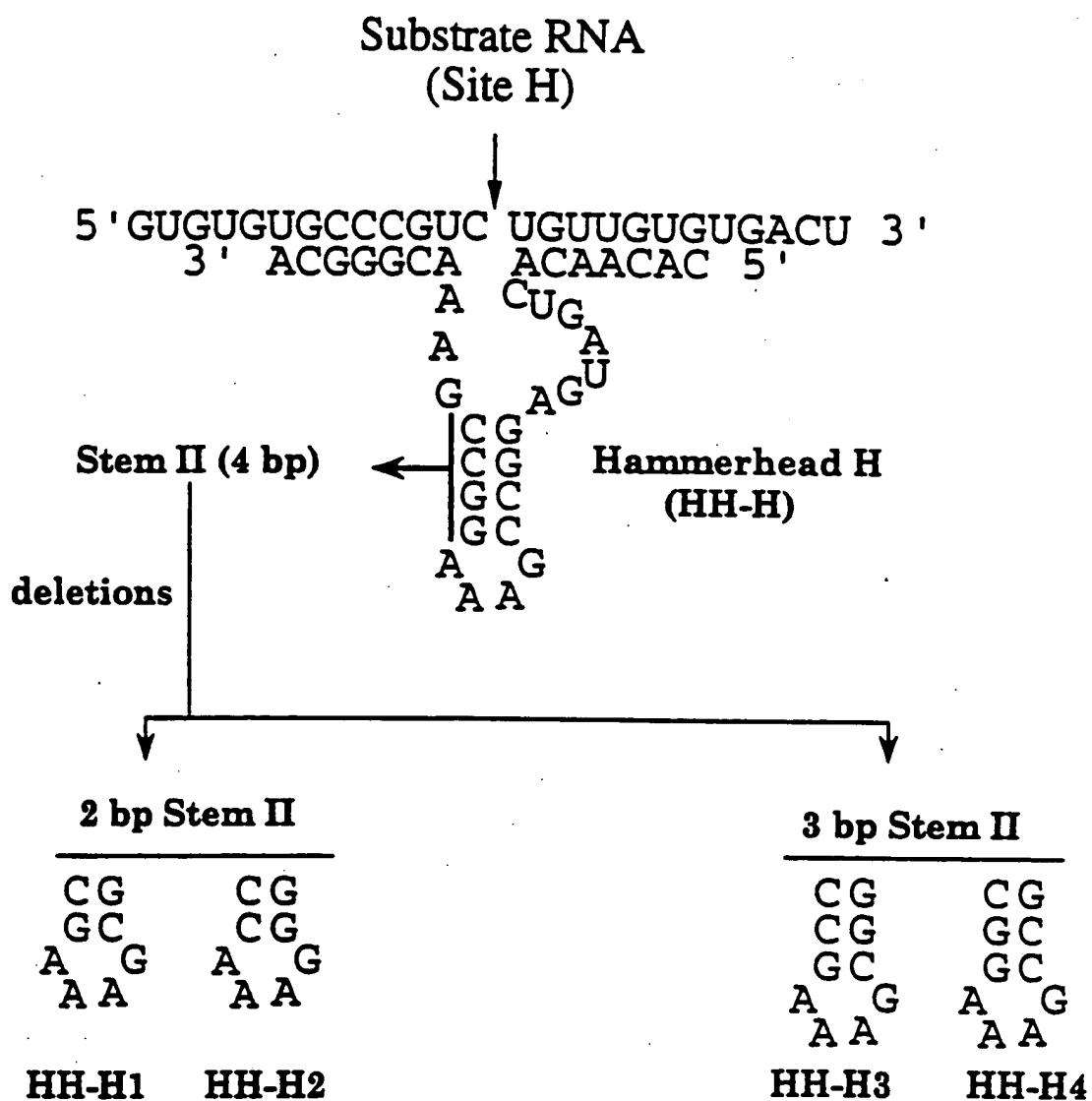
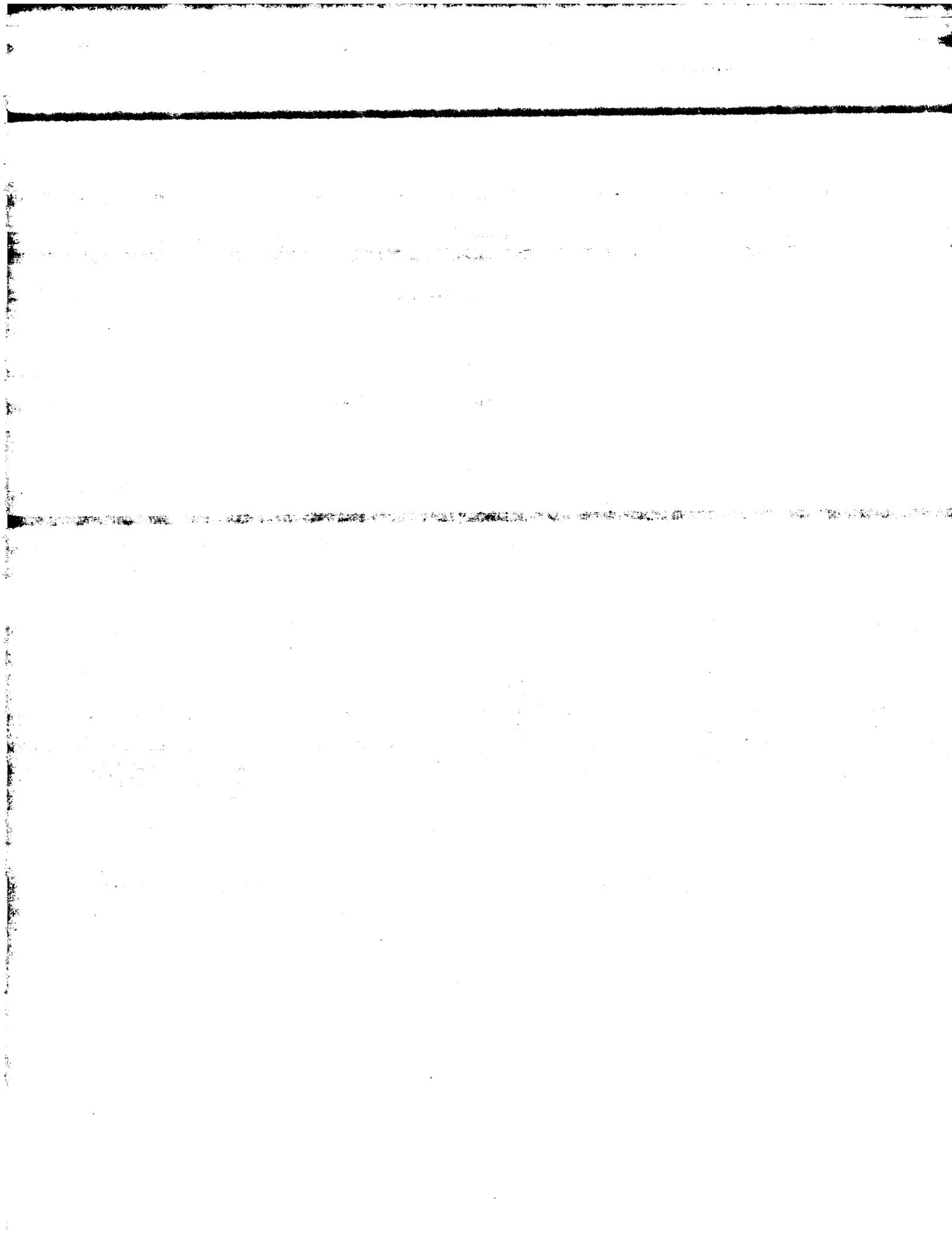


FIG. 62.



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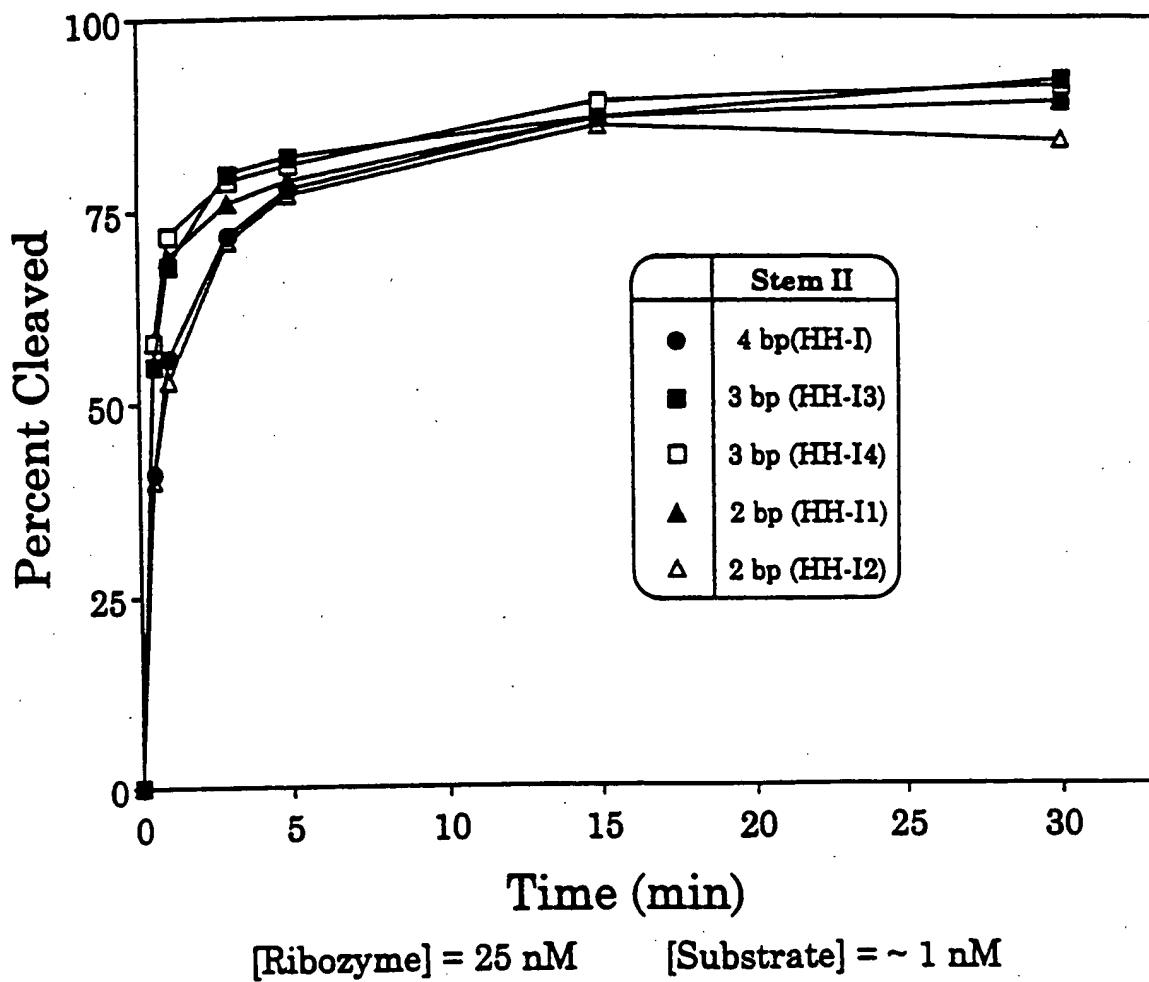
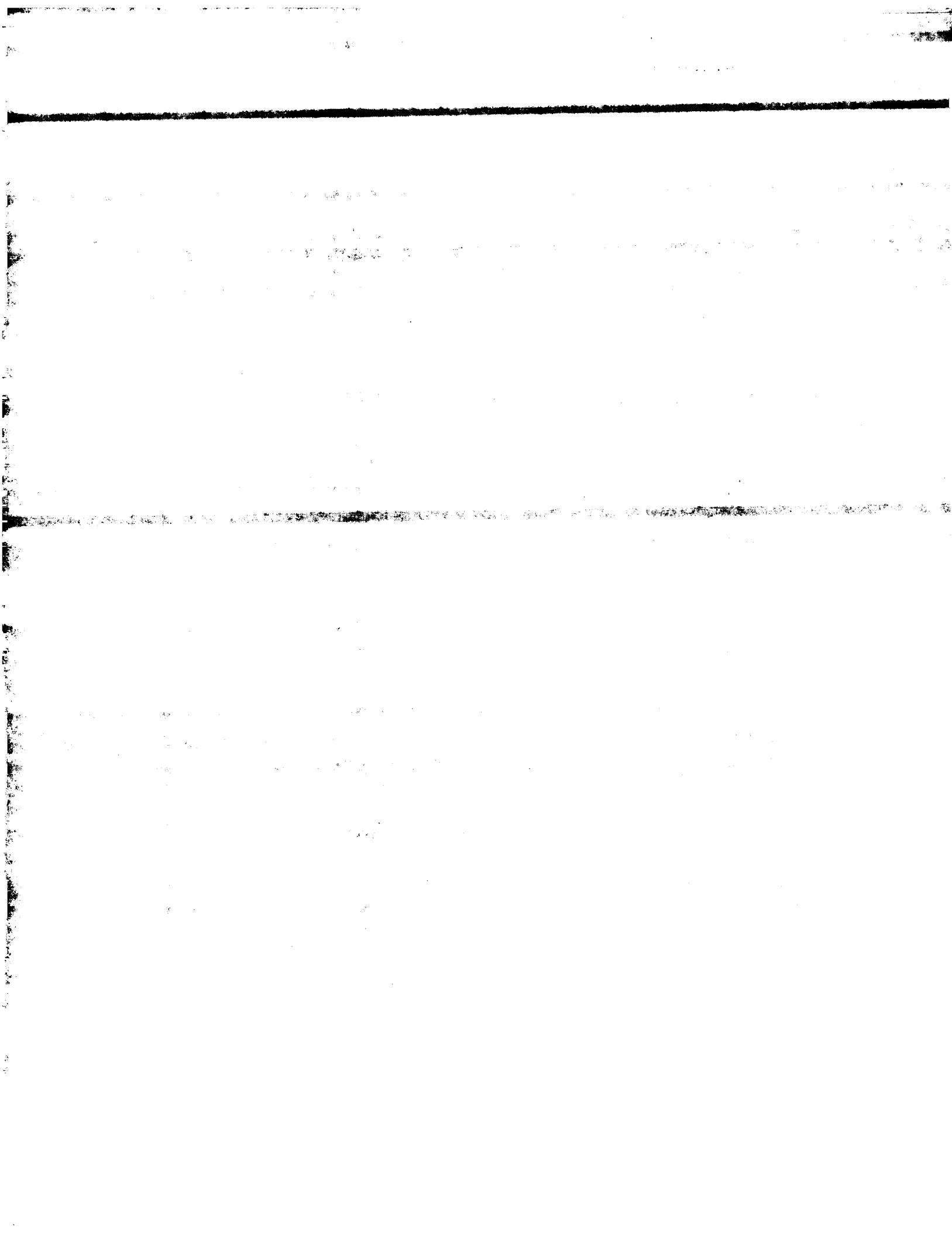


FIG. 63.



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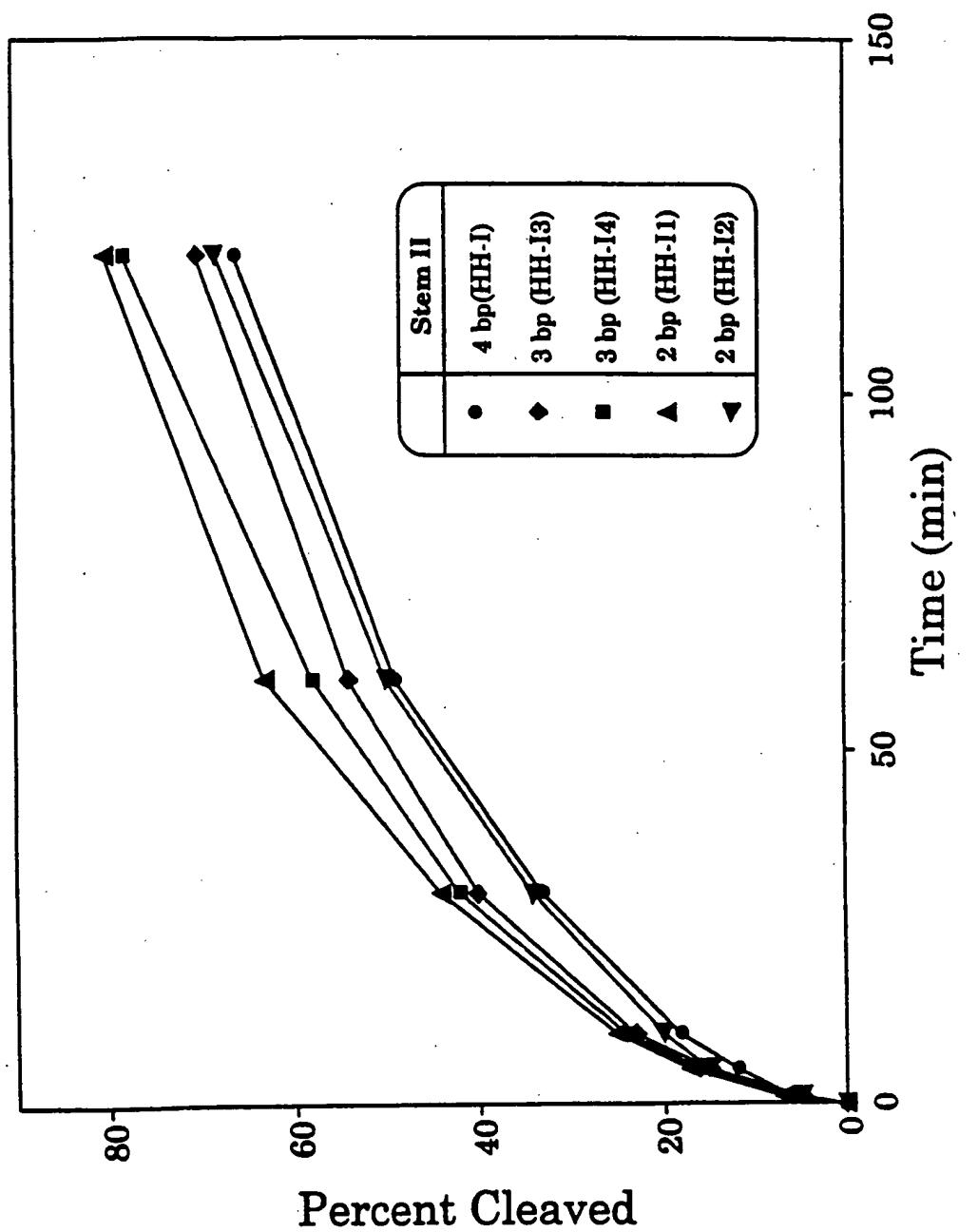


FIG. 64.

Fig. 65a.
Substrate RNA (site J)

Substrate RNA (site J)

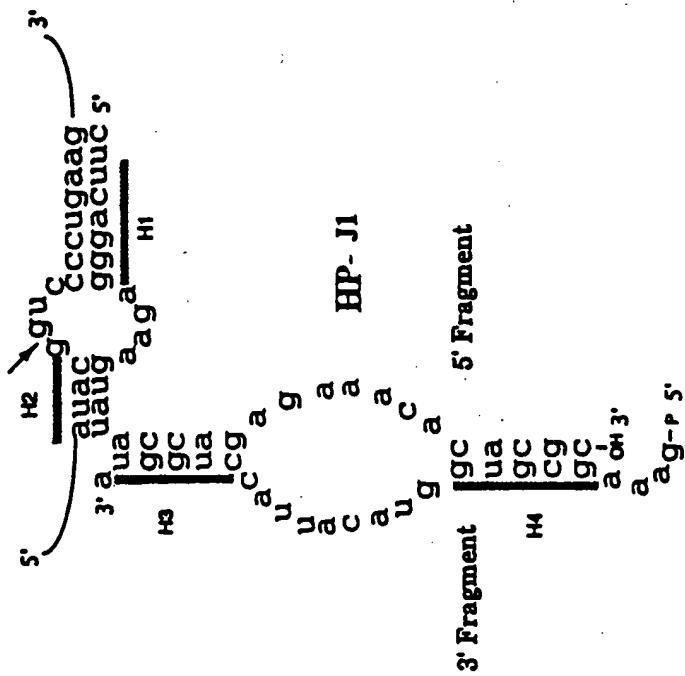
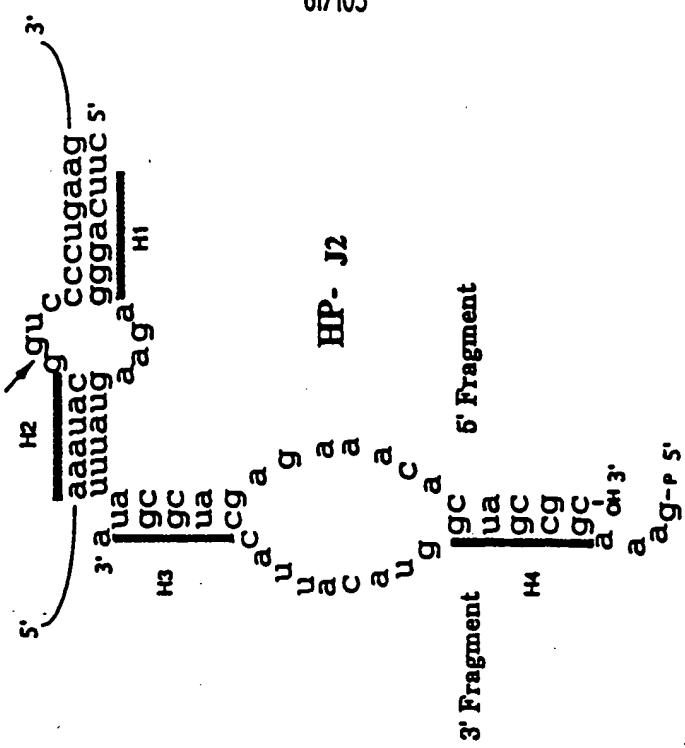
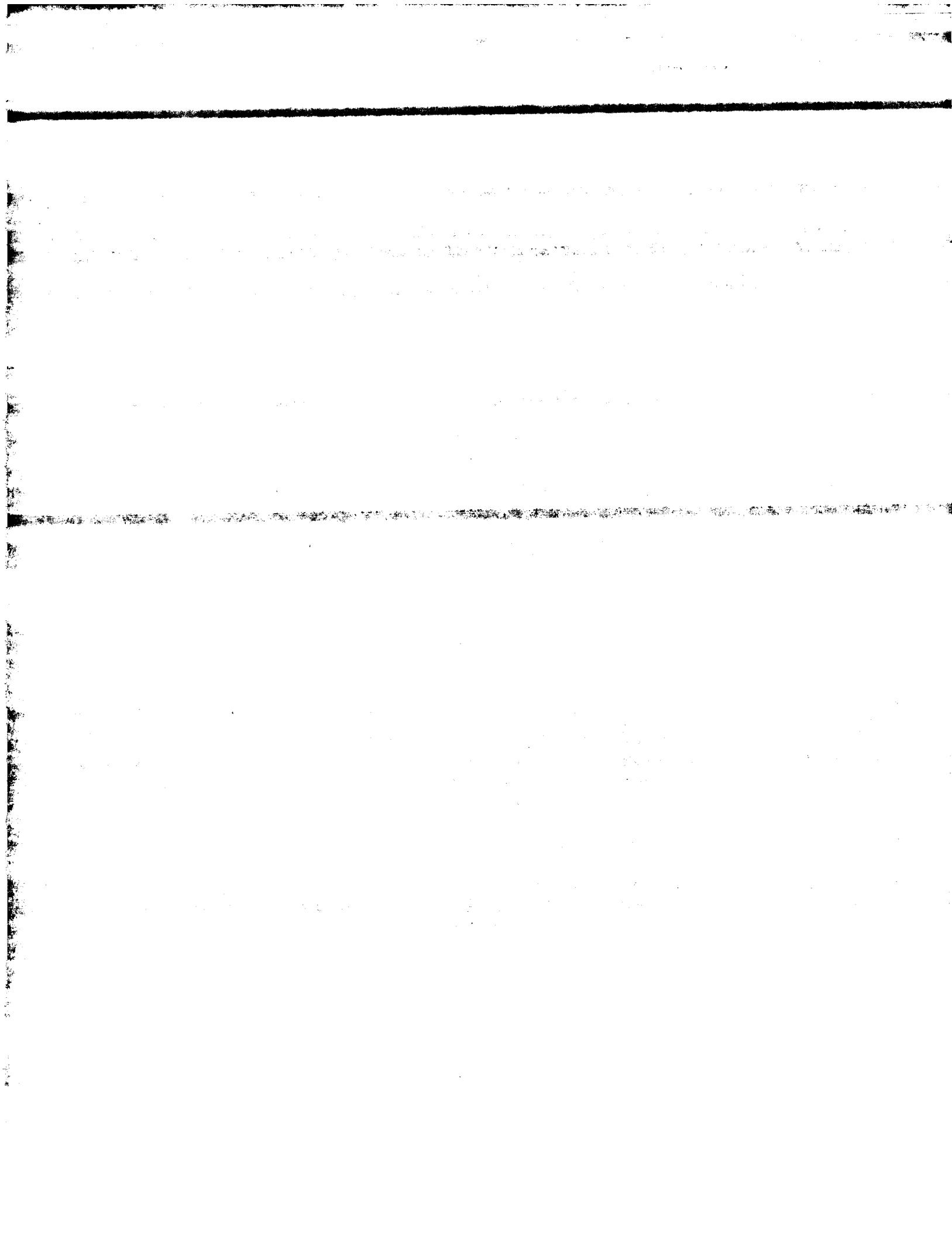


FIG. 65b.

Substrate RNA (site J)



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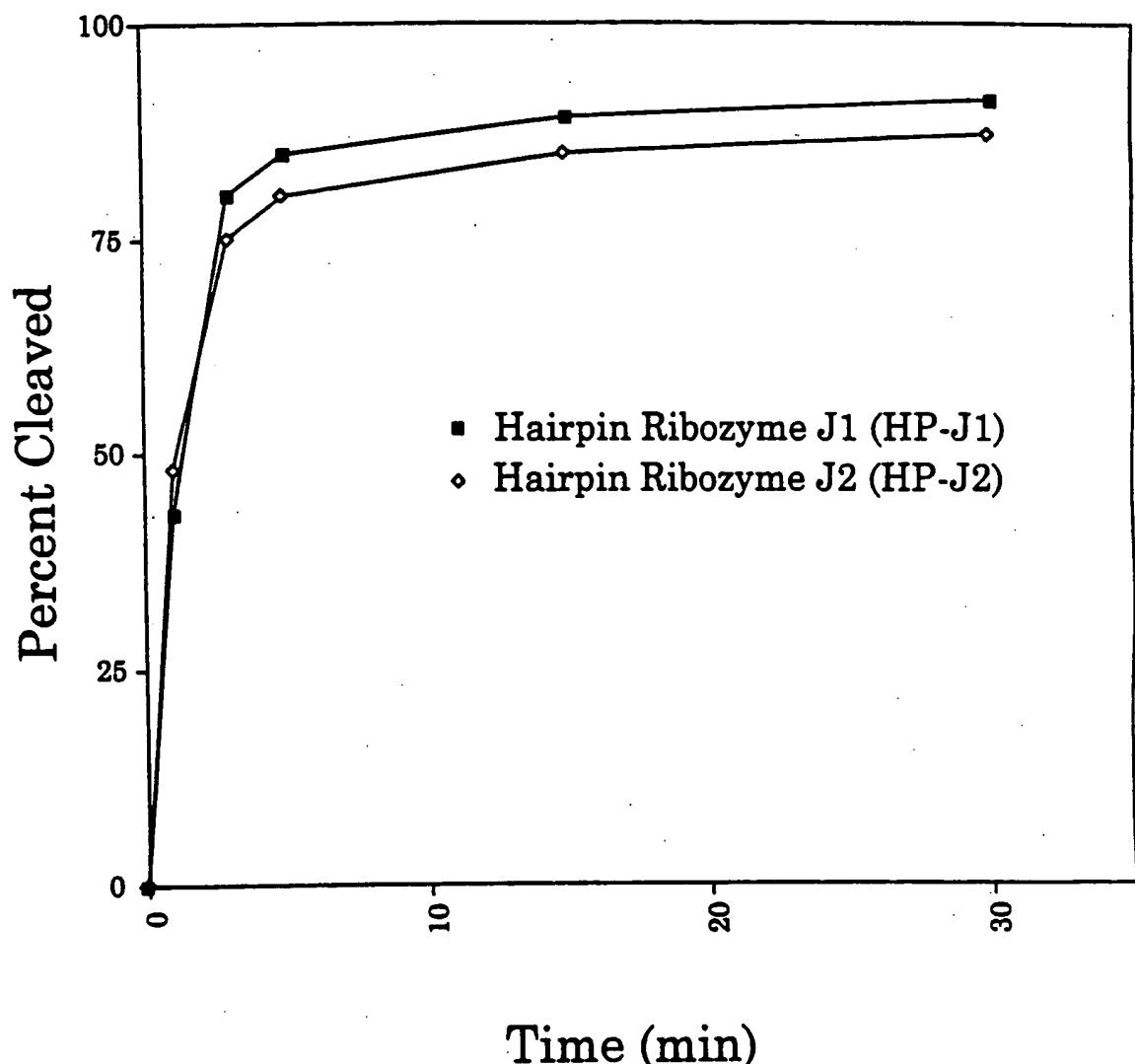
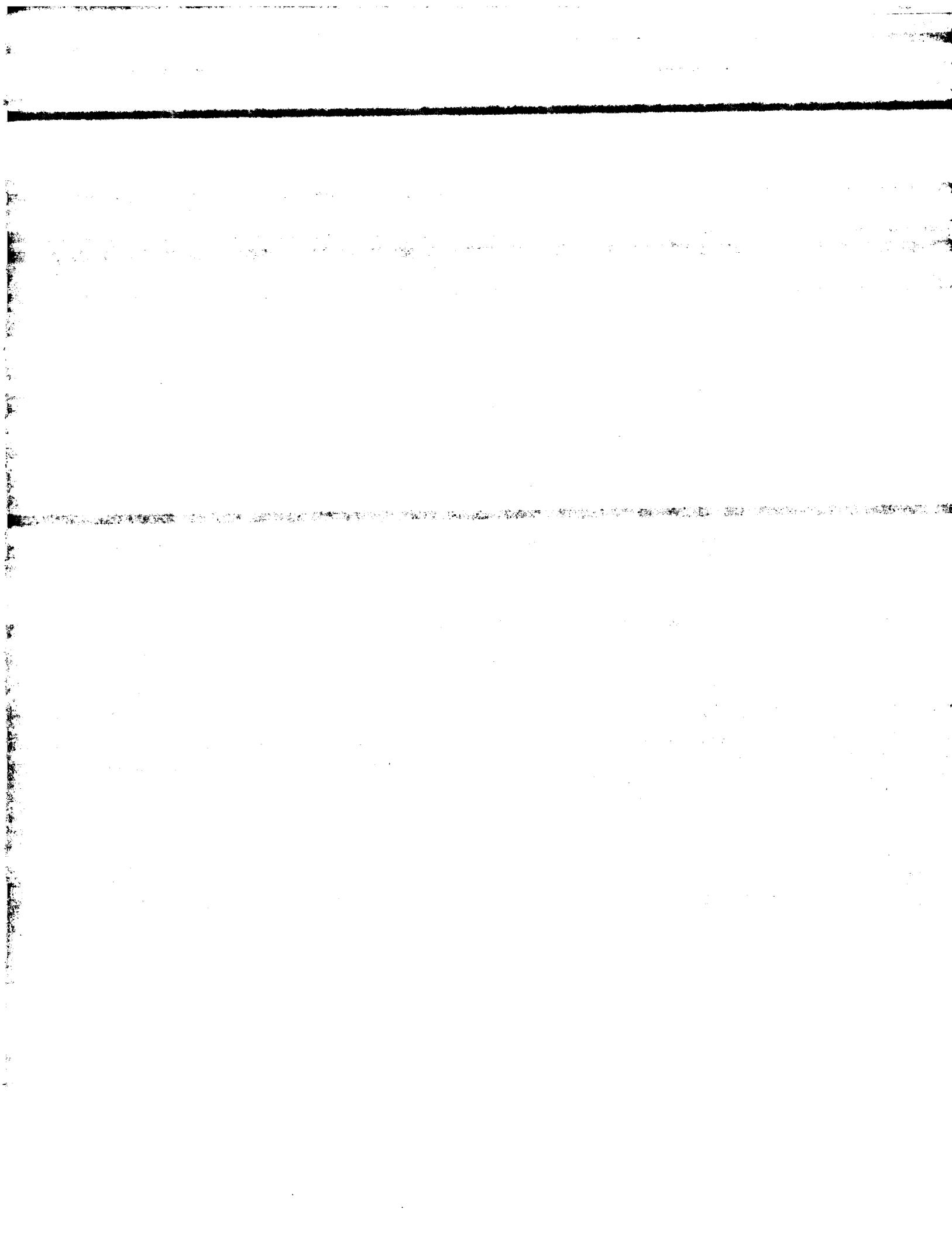


FIG. 66.



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FIG. 67b.

Substrate RNA

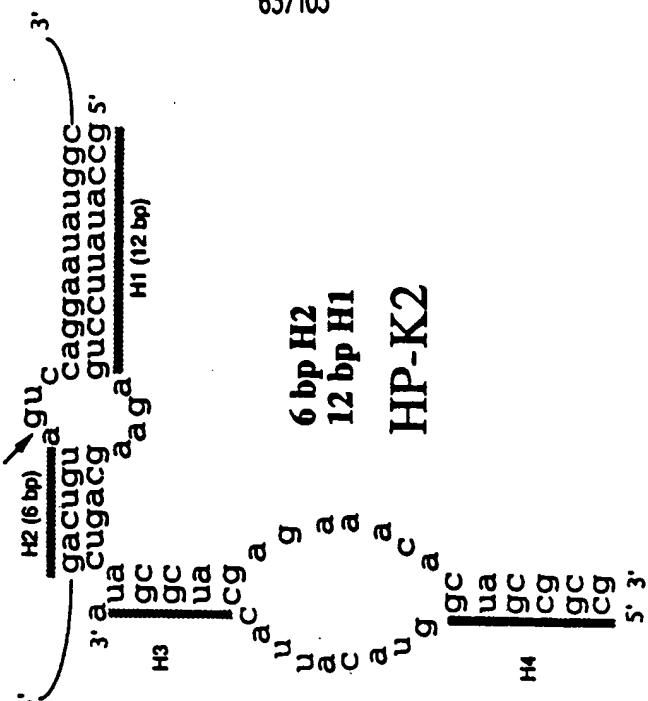
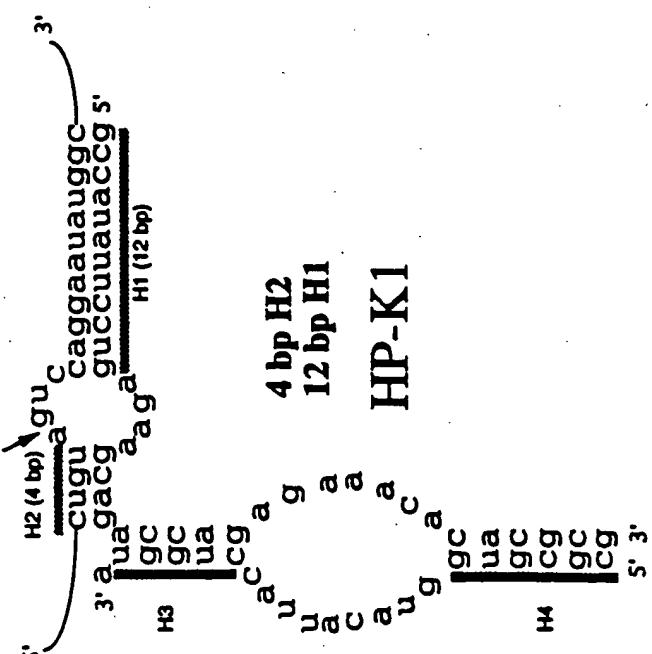
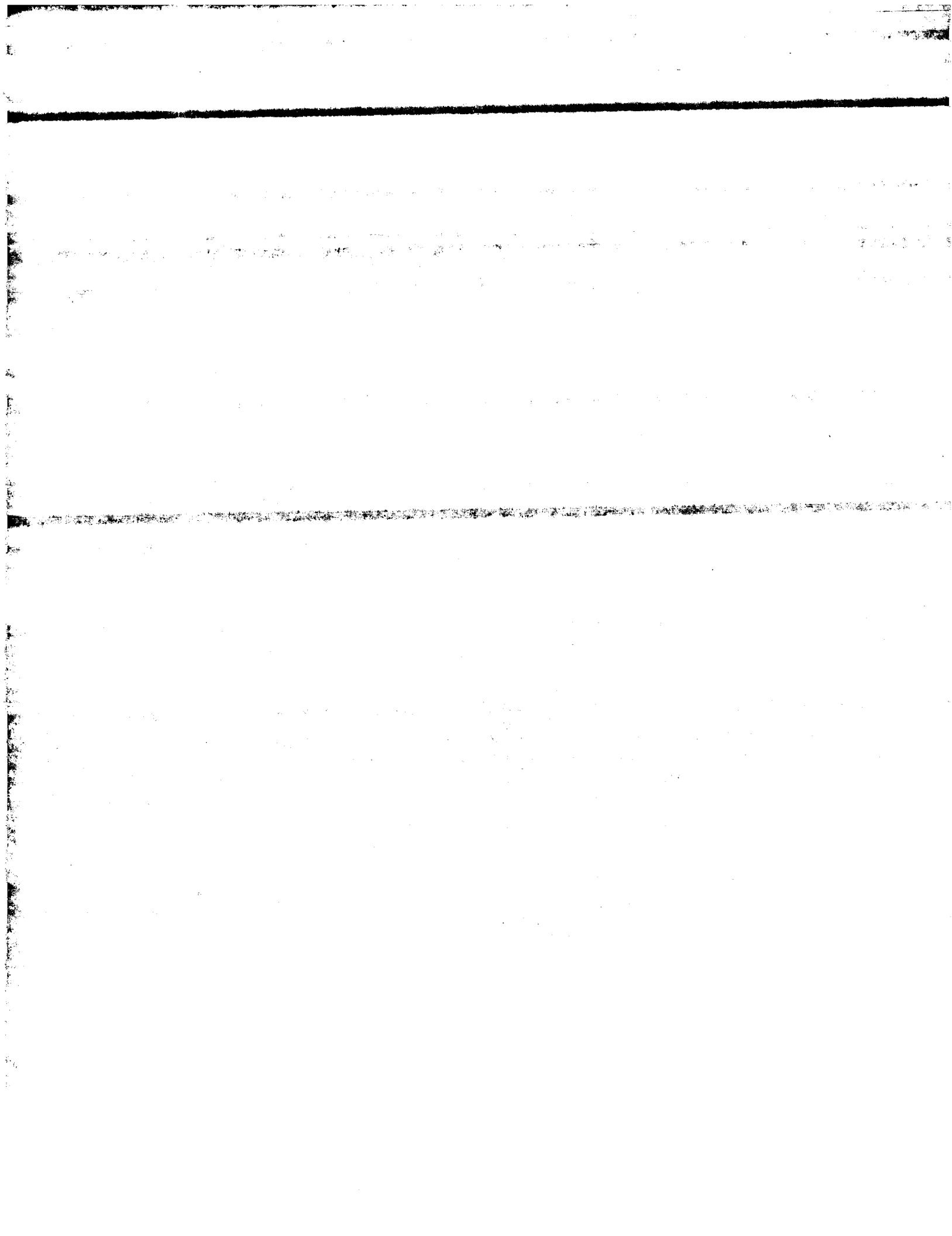


FIG. 67a.

Substrate RNA





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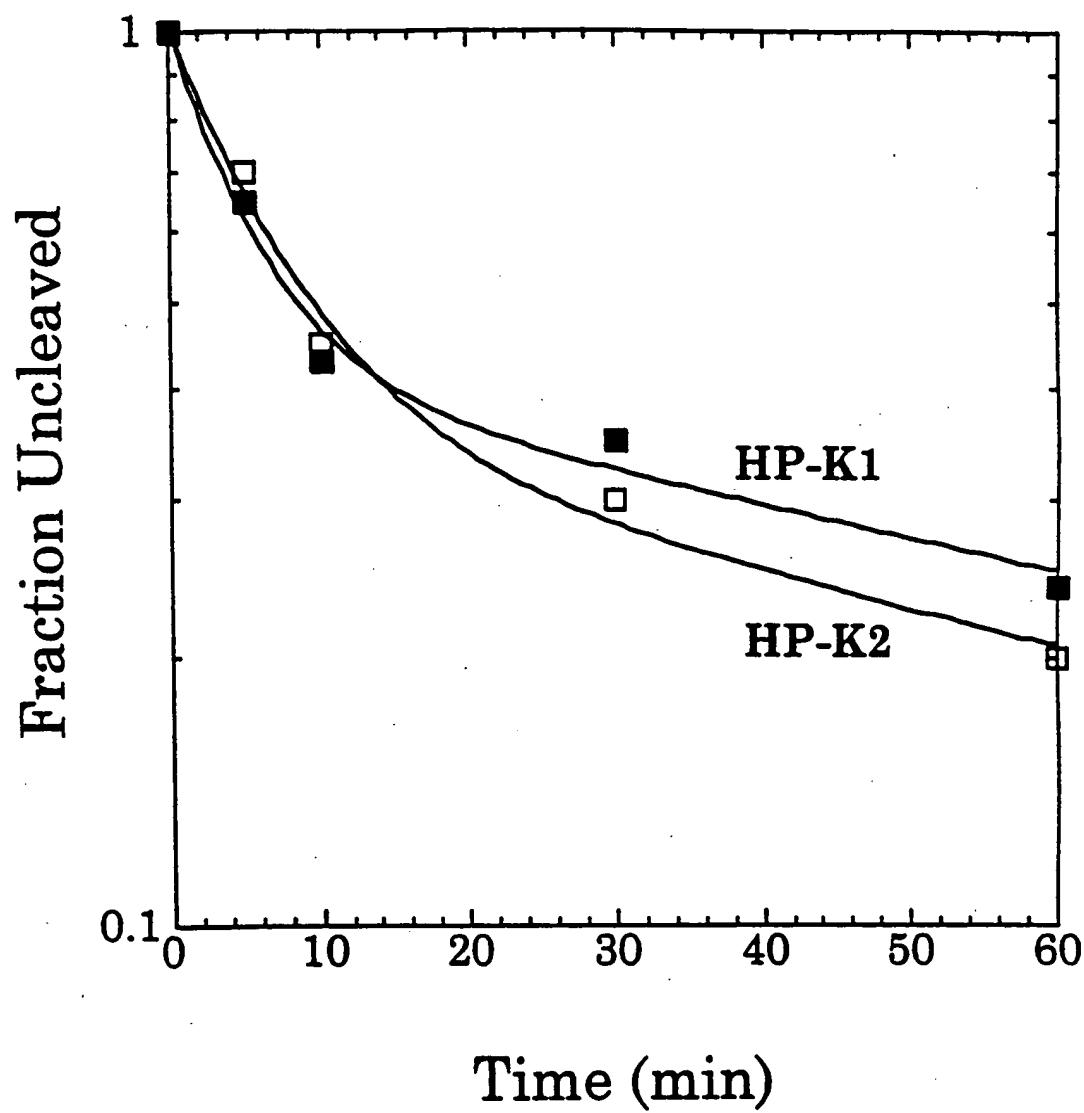
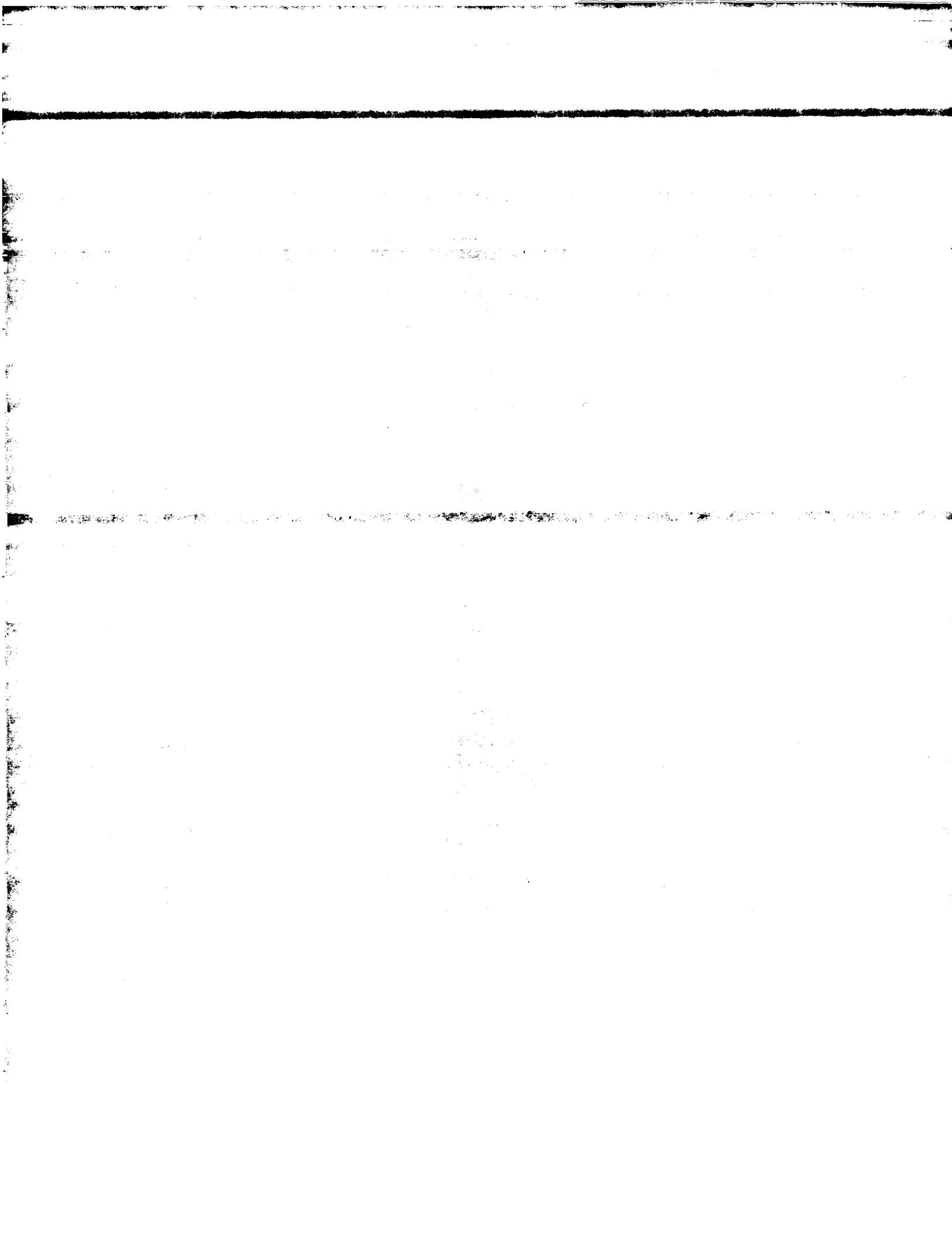


FIG. 68.



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FIG. 69b.

Substrate RNA

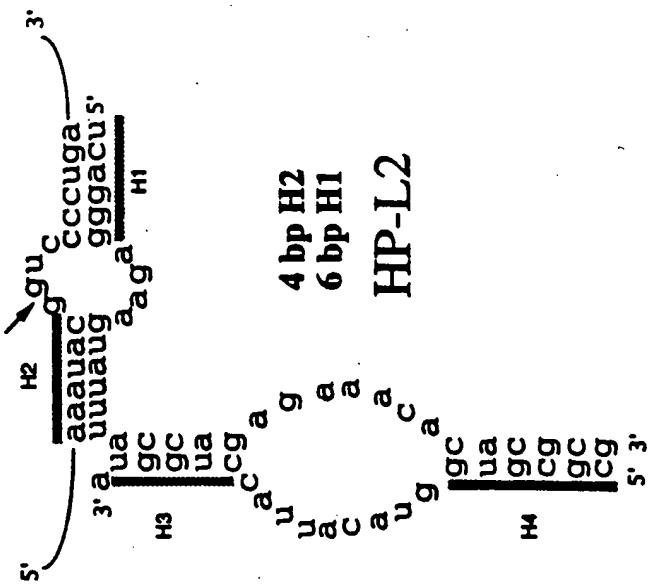
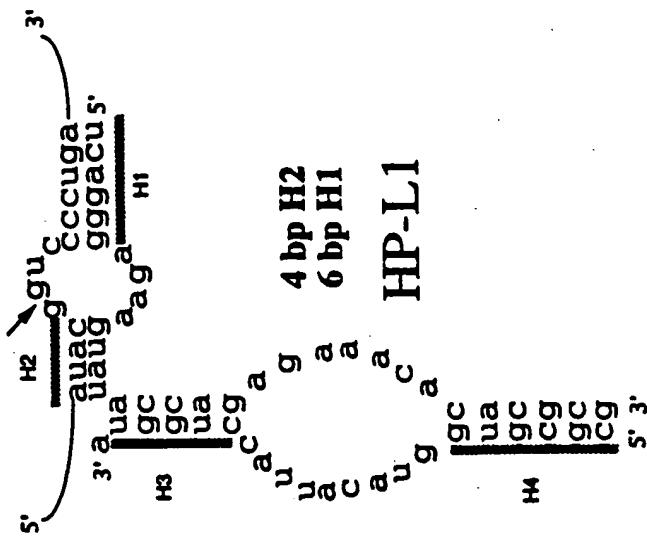
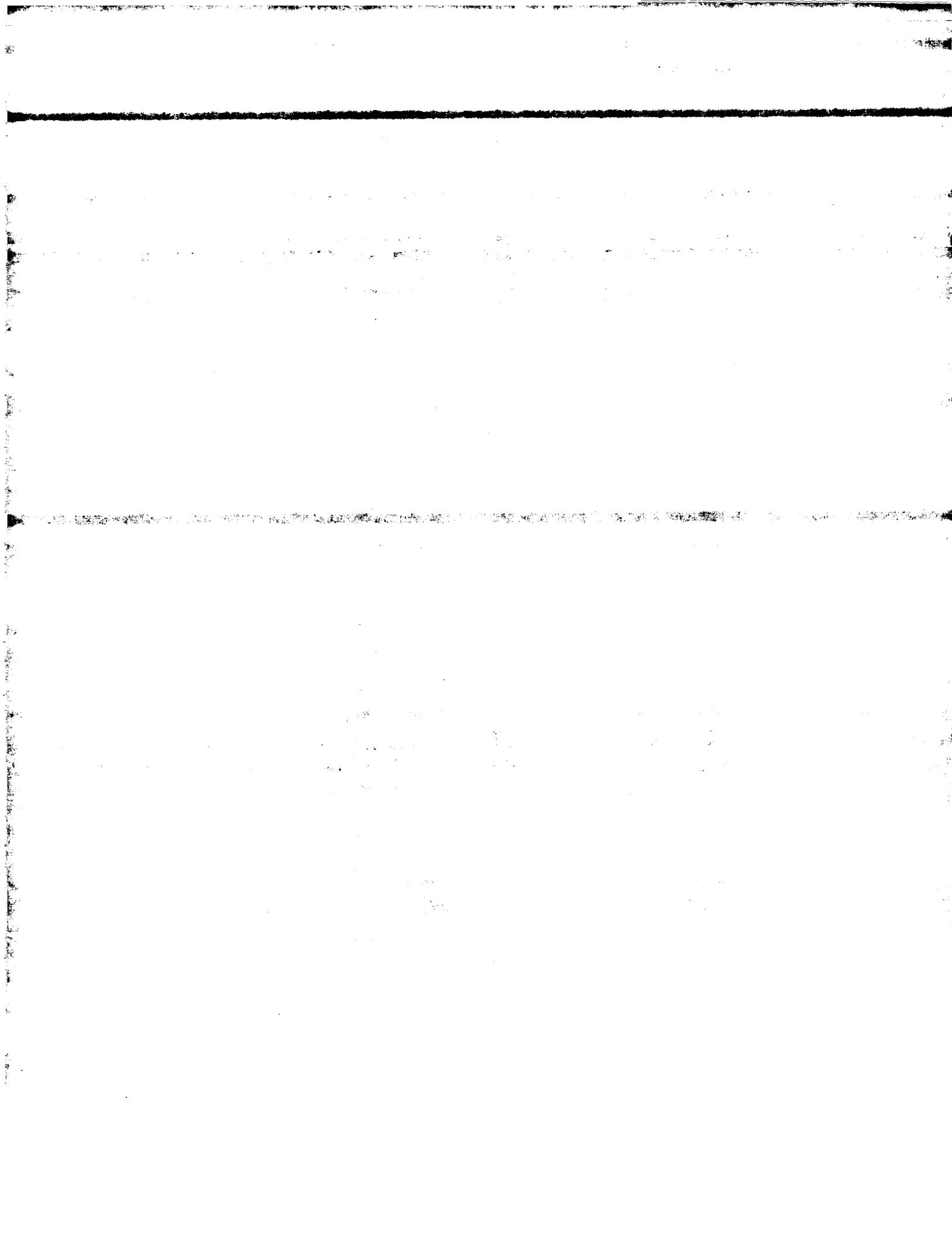


FIG. 69a.

Substrate RNA





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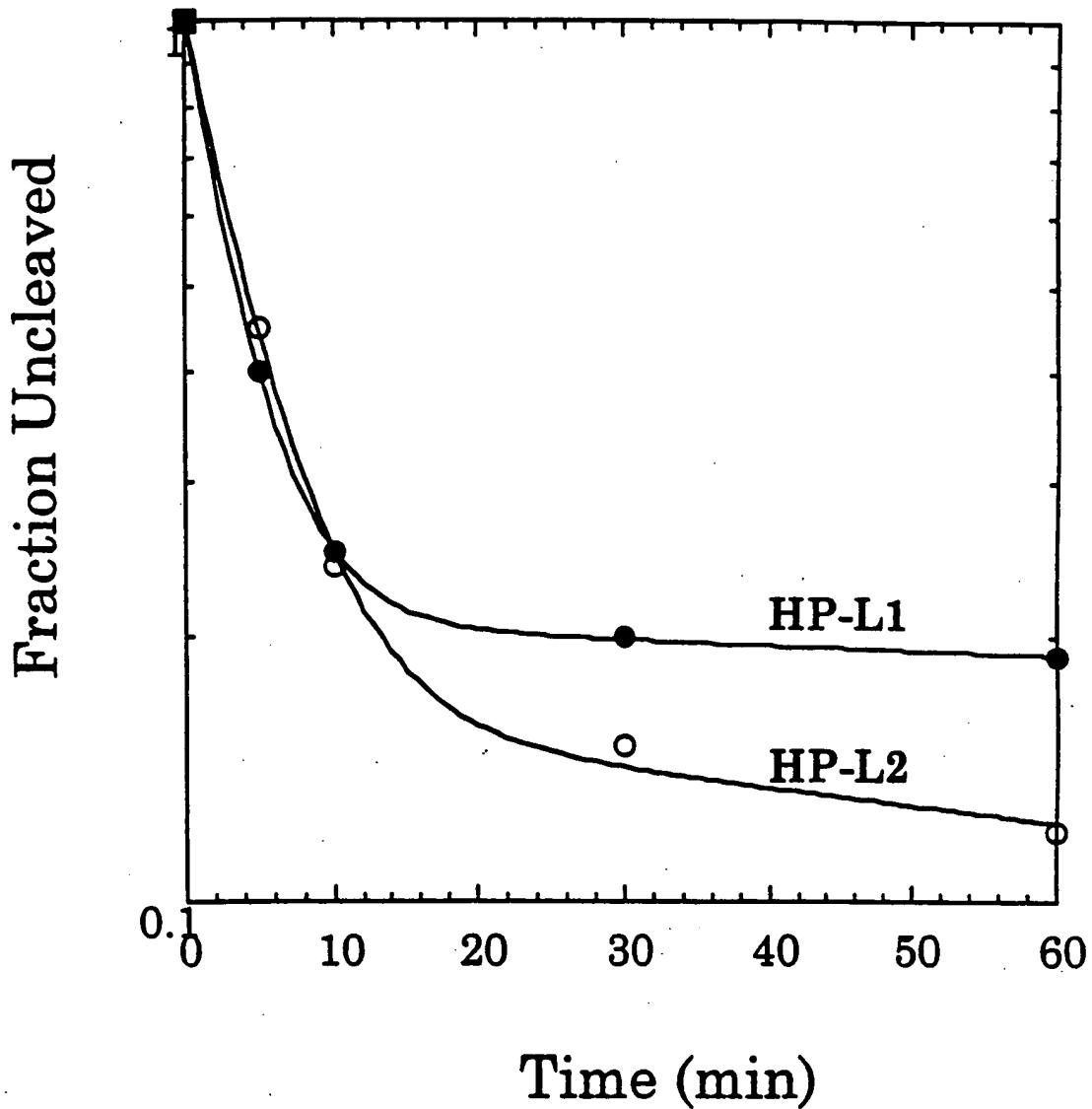


FIG. 70.

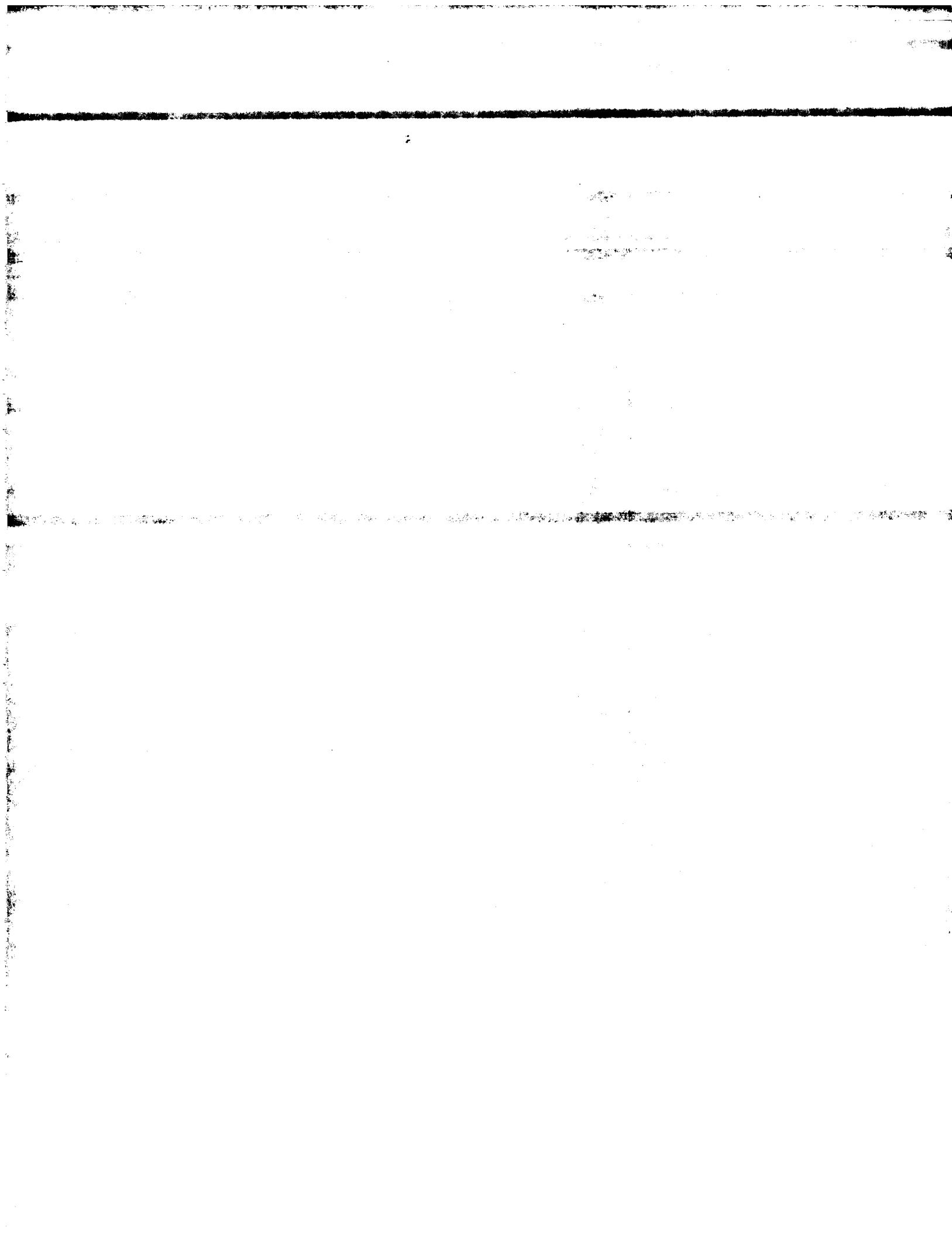


FIG. 7a.

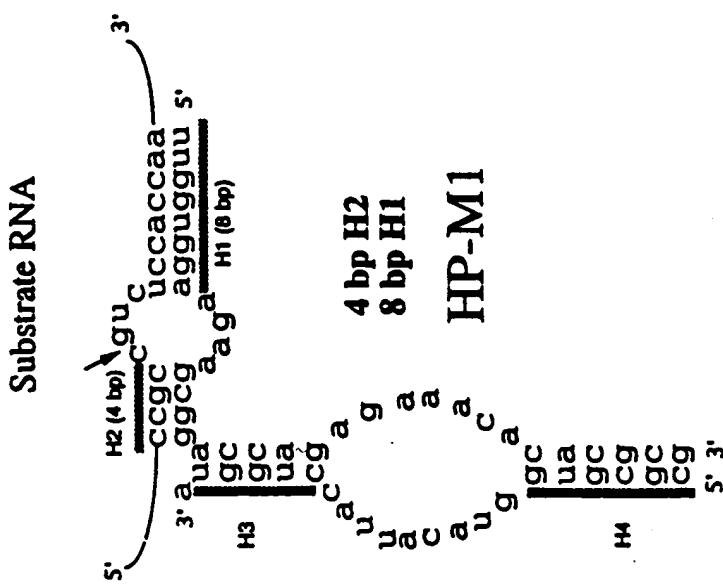
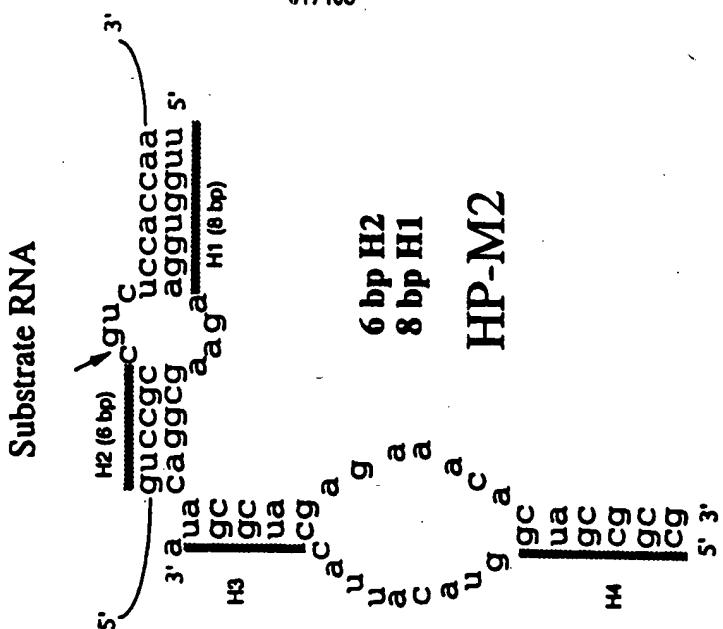
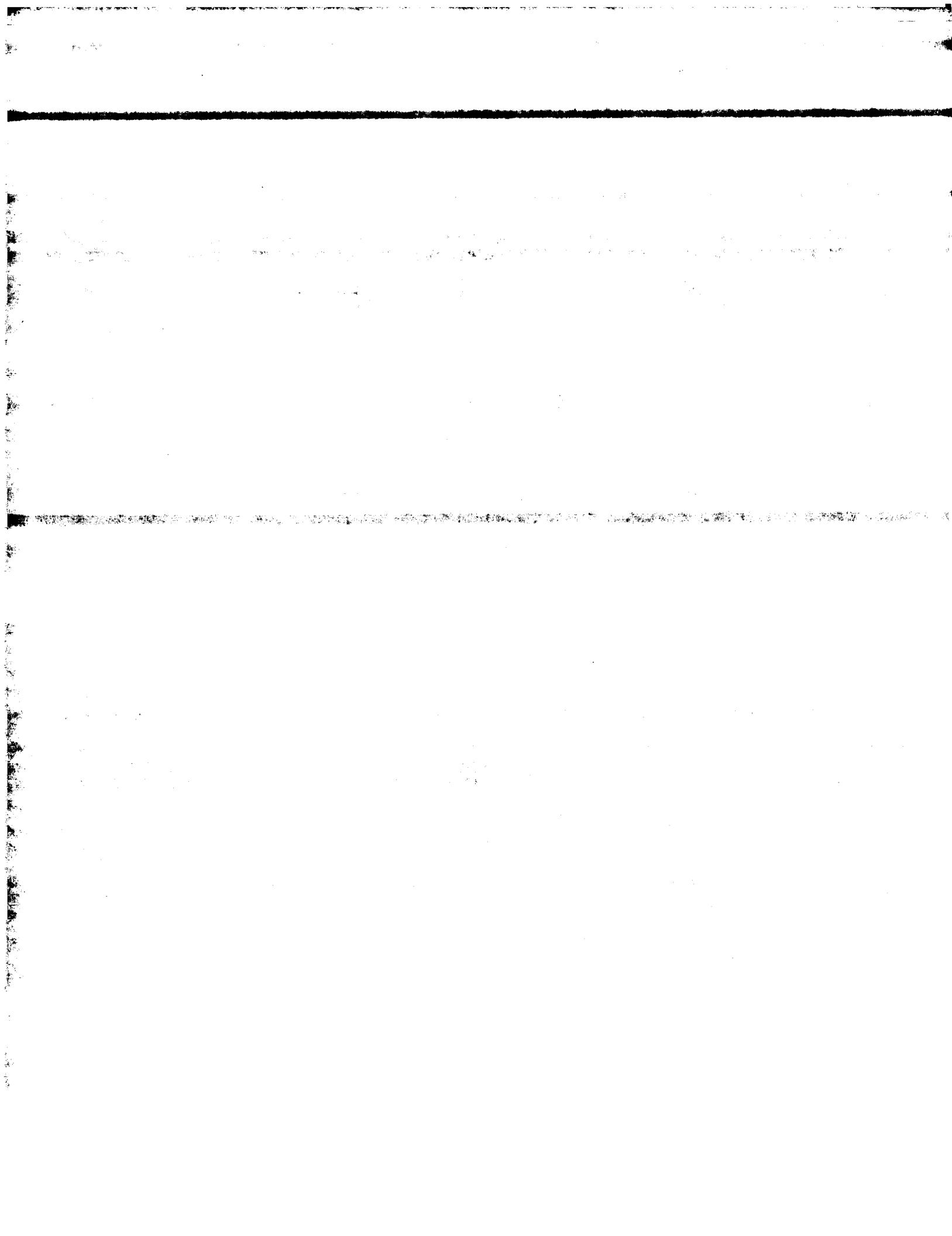


FIG. 7b.





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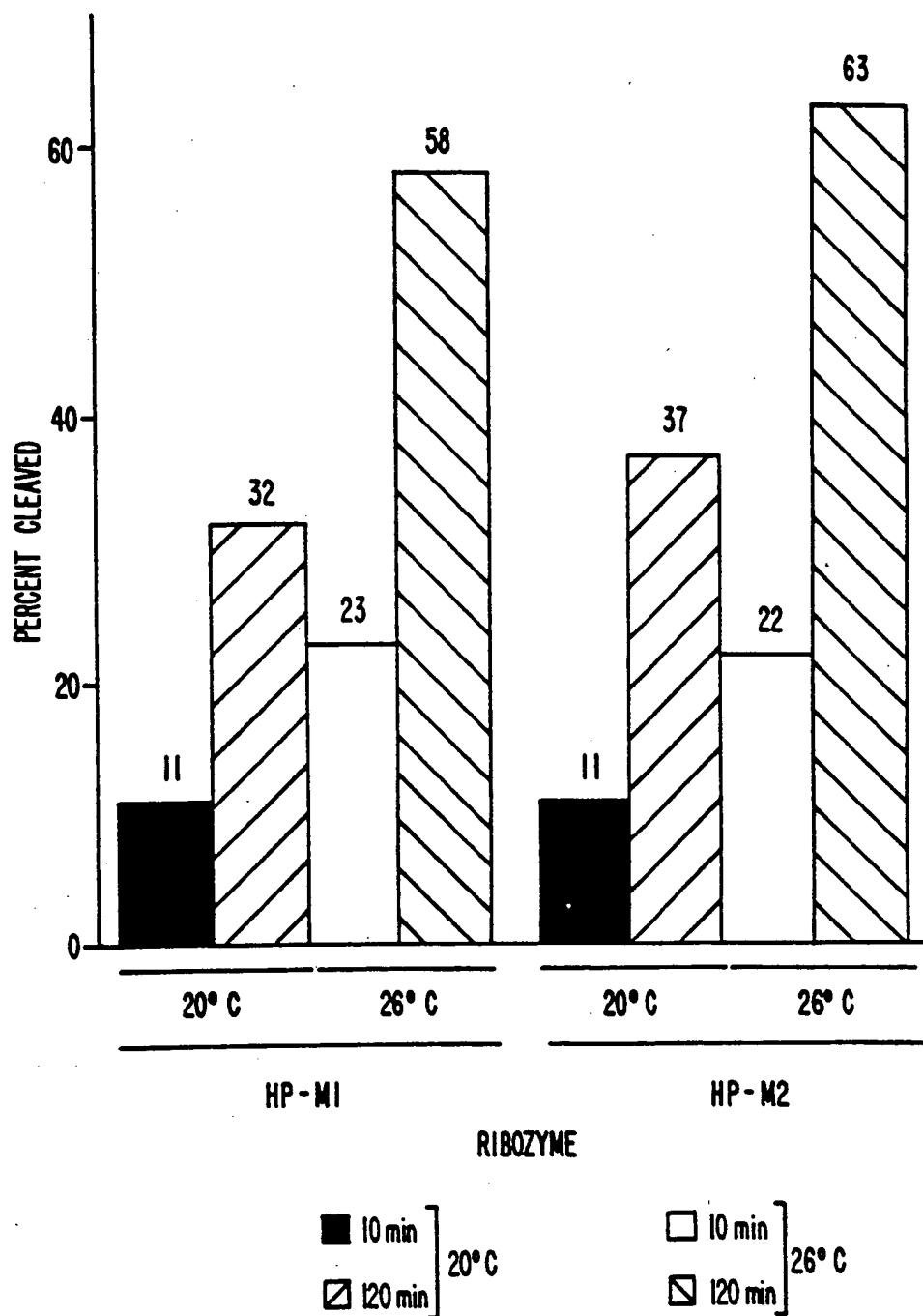
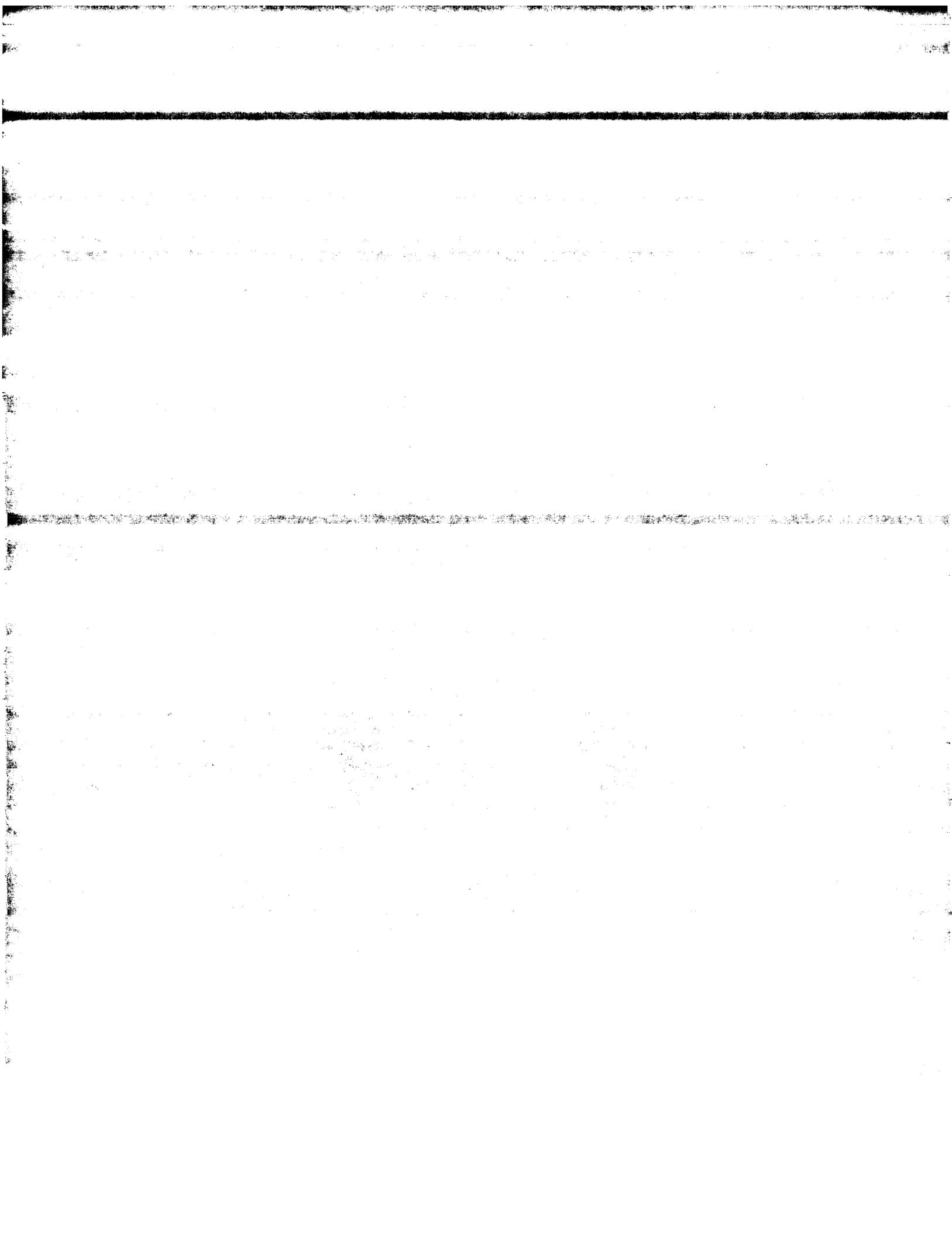


FIG. 72.



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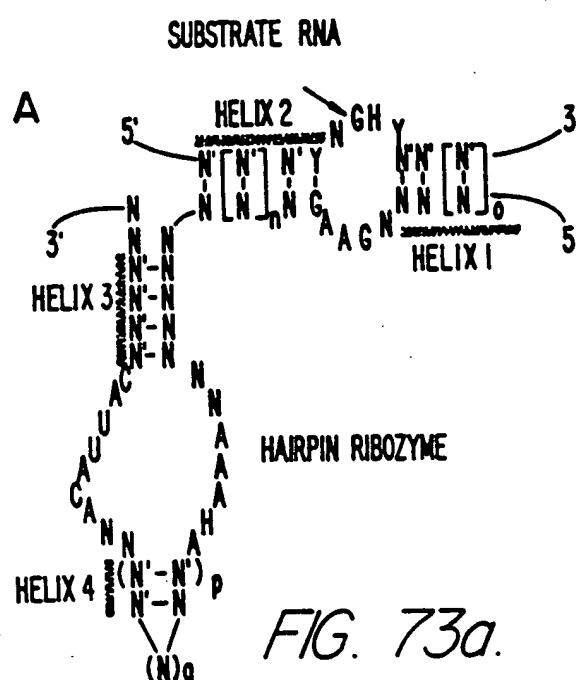


FIG. 73a.

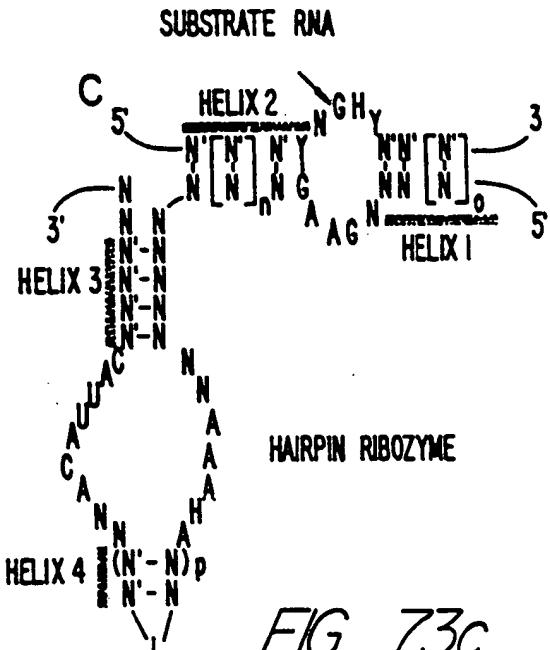


FIG. 73c.

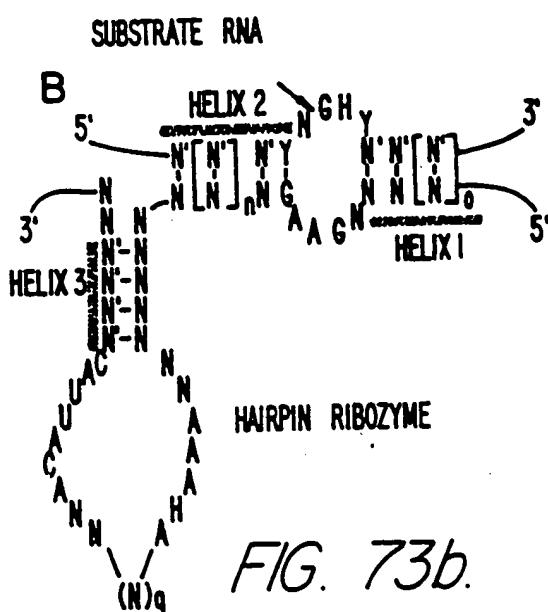


FIG. 73b.

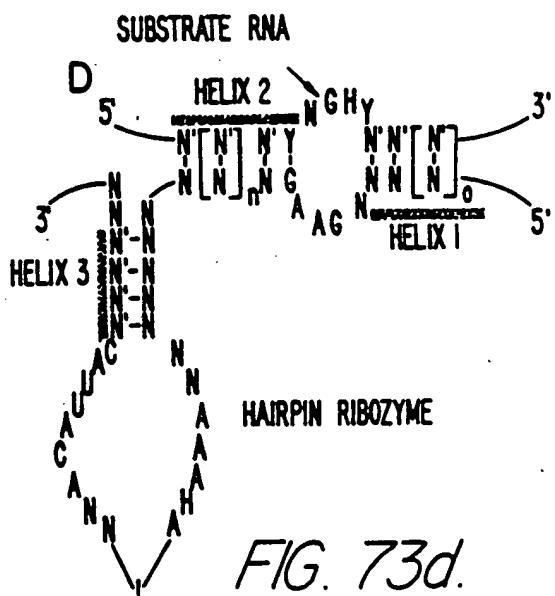
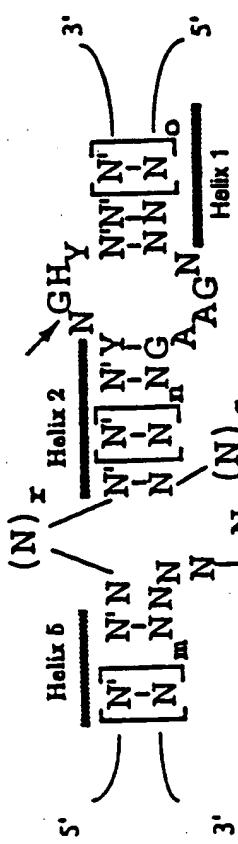


FIG. 73d.

FIG. 74a.

Substrate RNA



Hairpin Ribozyme

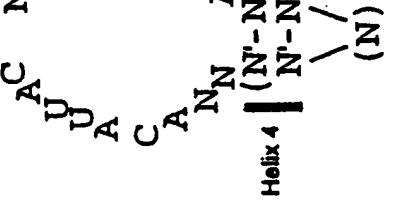
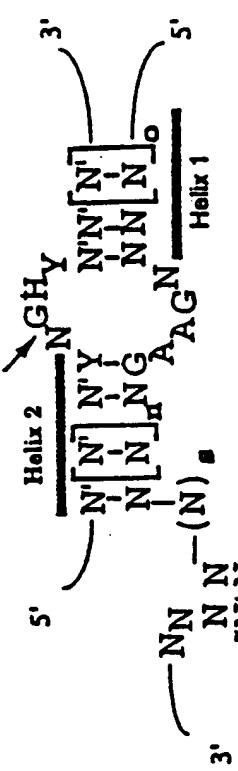


FIG. 74b.

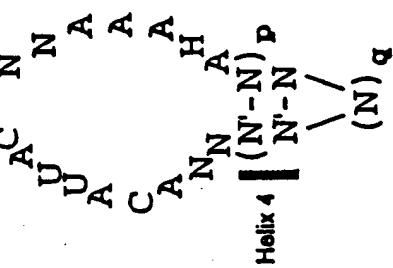
B

Substrate RNA



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Hairpin Ribozyme



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FIG. 75a.

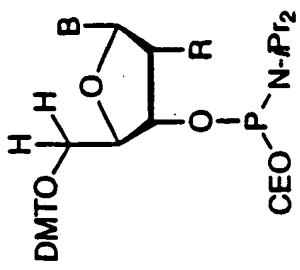


FIG. 75b.

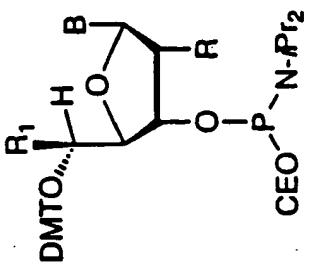
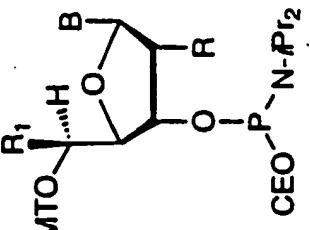


FIG. 75c.



1

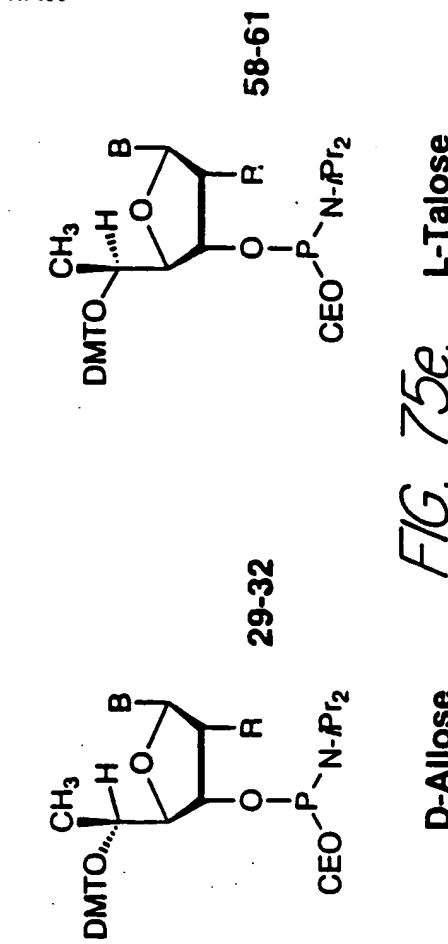
D-Ribose Family

2

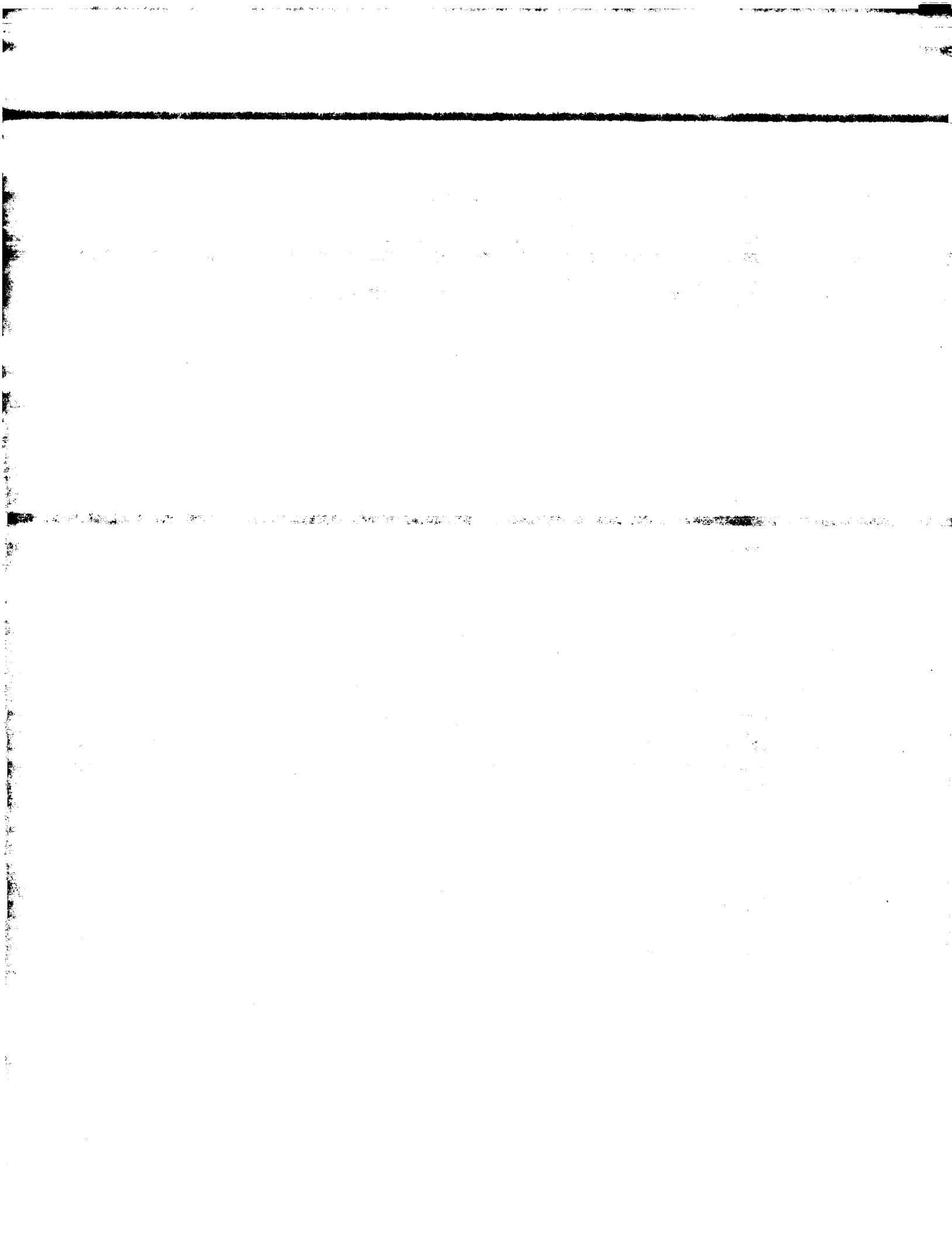
D-Allose Family

3

L-Talose Family



B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.



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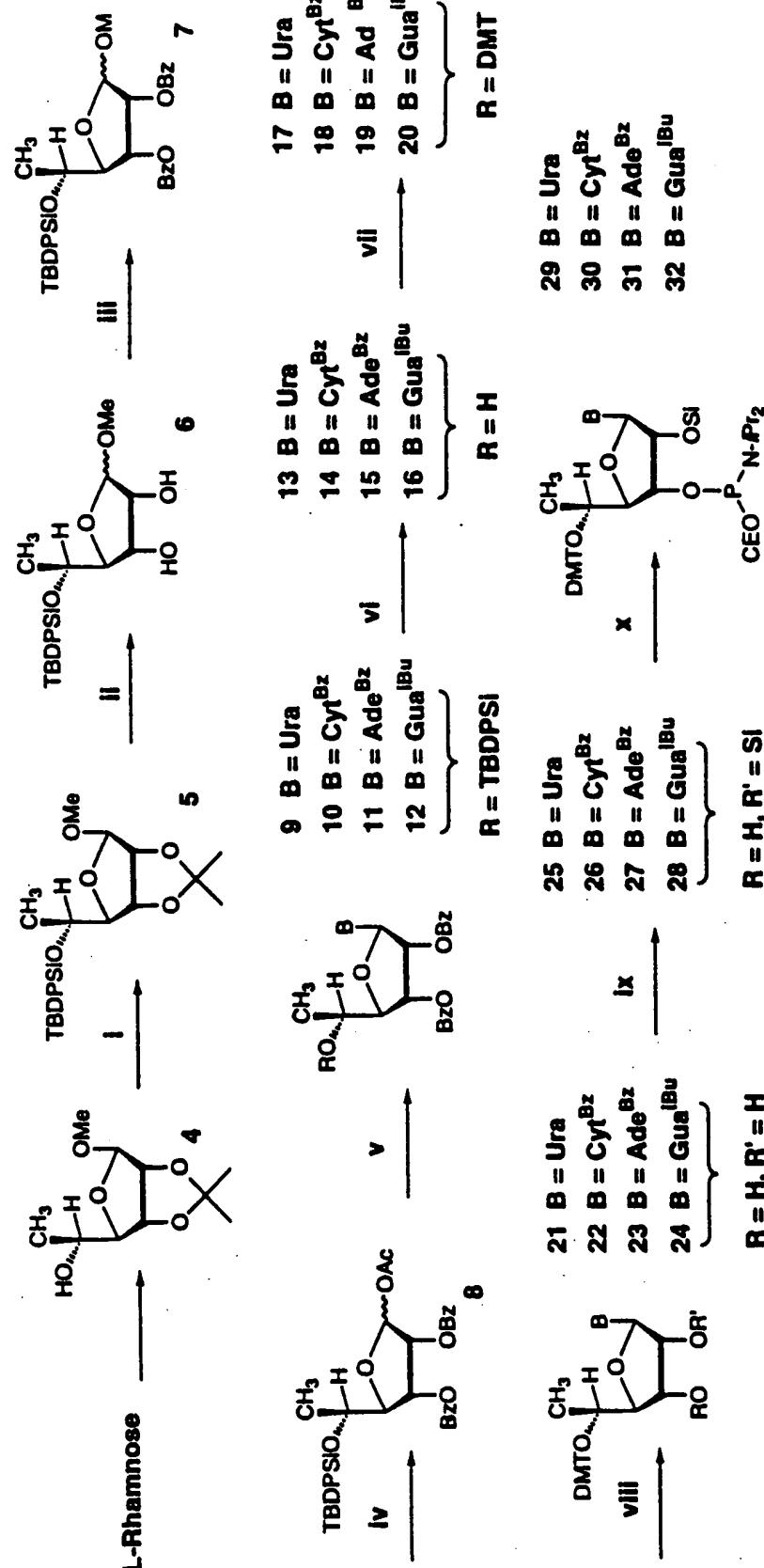
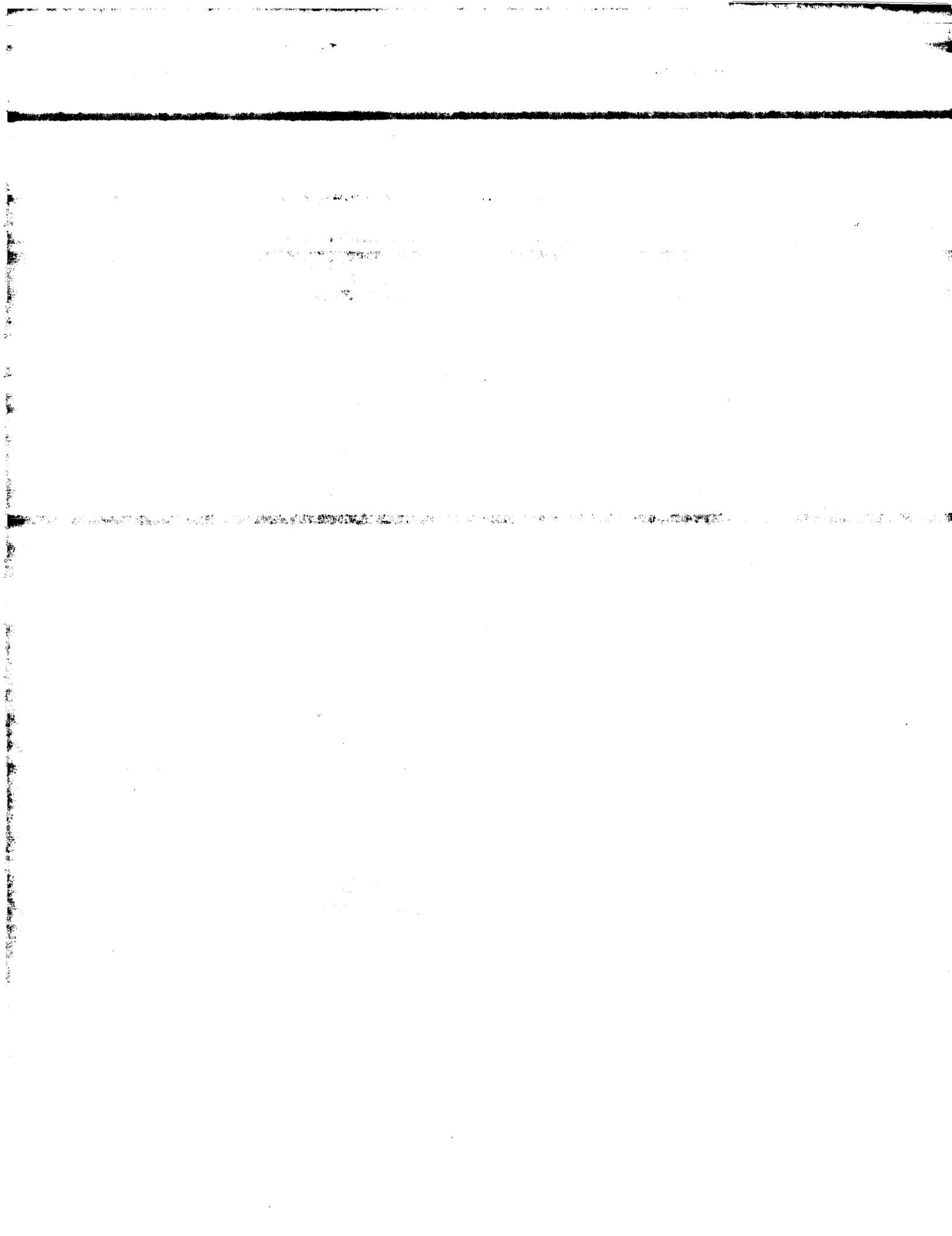
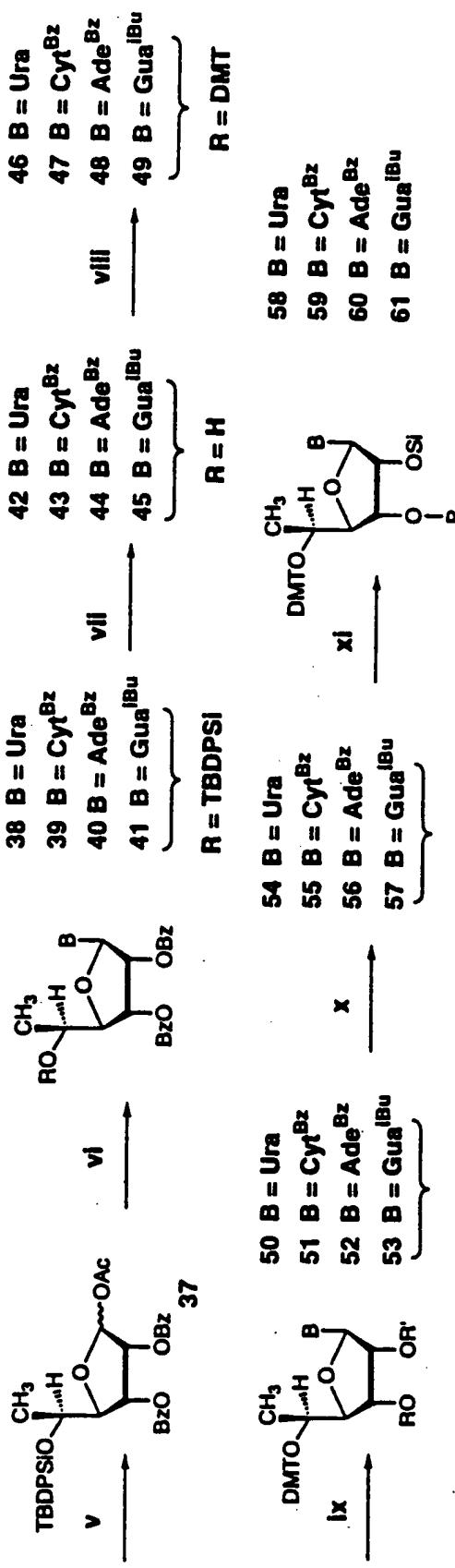
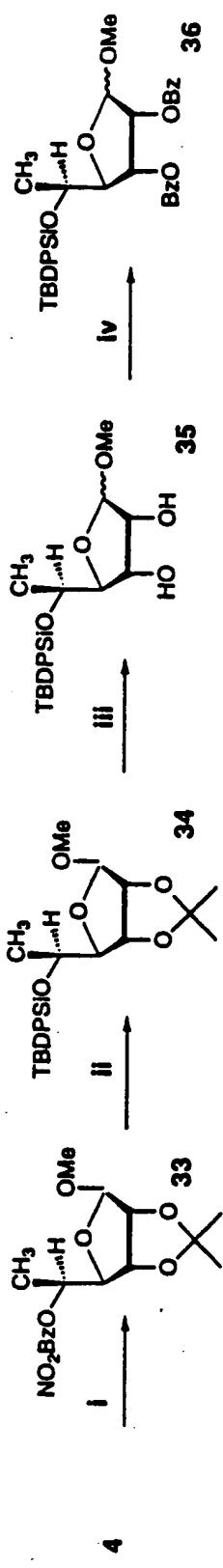


FIG. 76.



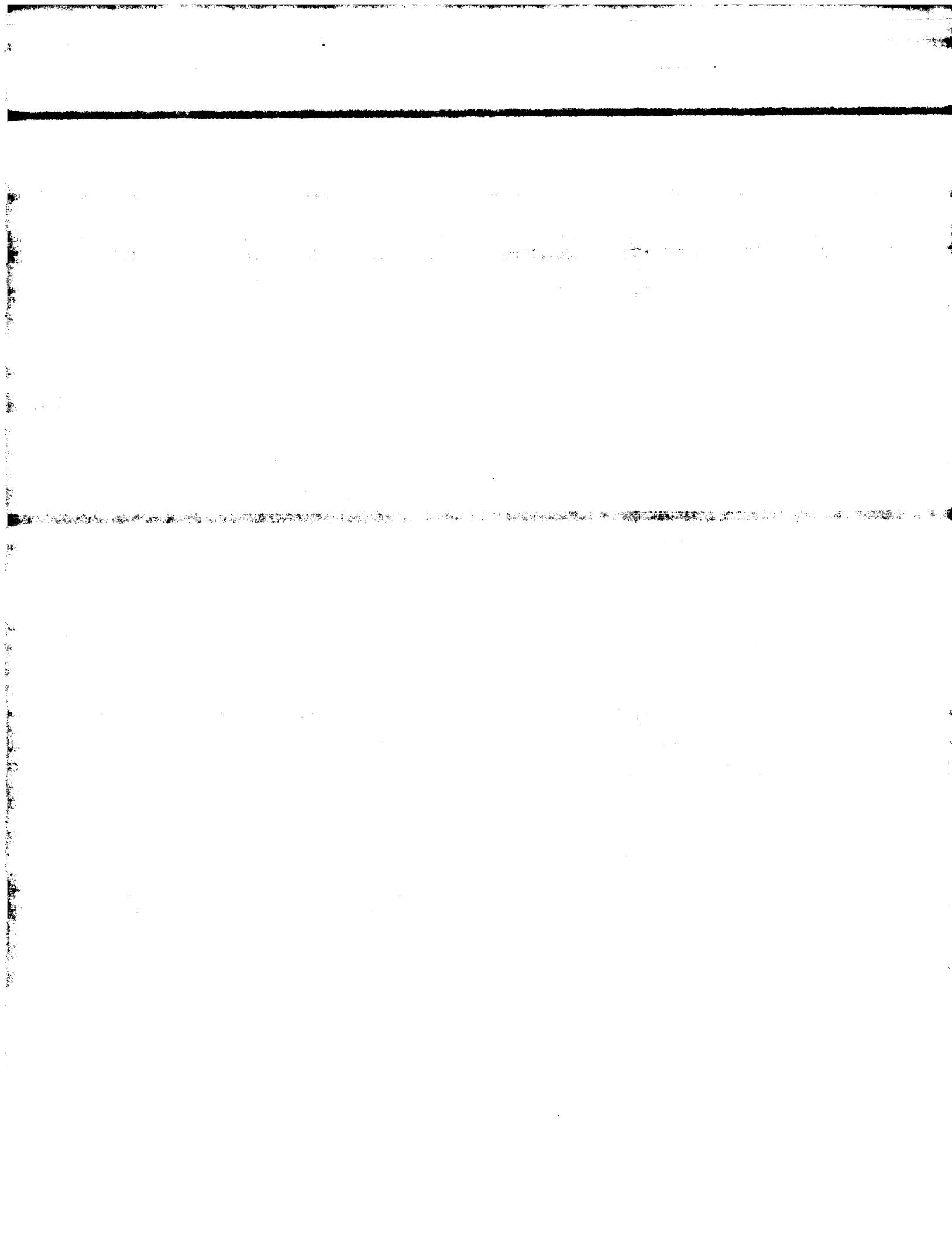
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i) = Ph₃P/DEAD/p-NO₂PhCOOH
ii) = OH⁻, TBDPSSi-Cl
iii) = H⁺
iv) = Bz-Cl/Pyr

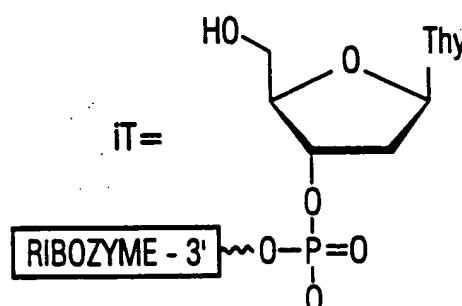
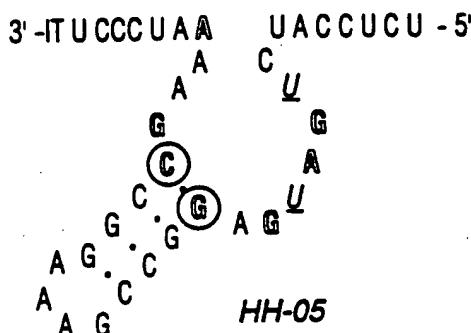
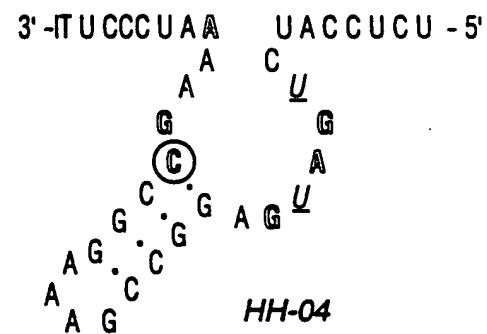
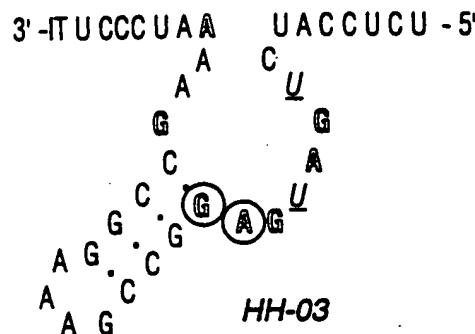
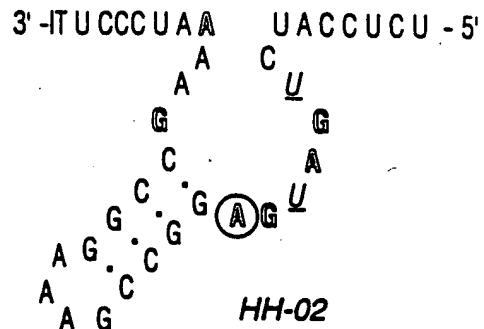
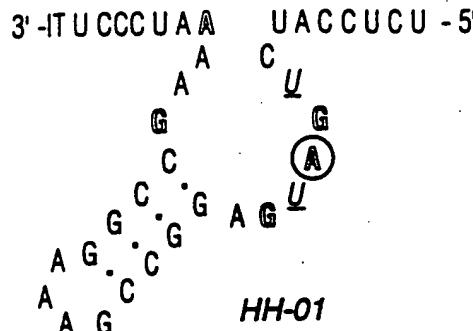
v) = AcOH/Ac₂O/H⁺
vi) = B^{TMS}/CF₃SO₃SiMe₃
vii) = TBAF
viii) = DMT-Cl/AgNO₃

ix) = OH⁻
x) = TBDMSi-Cl
xi) = P(OCE)(N-iPr₂)Cl



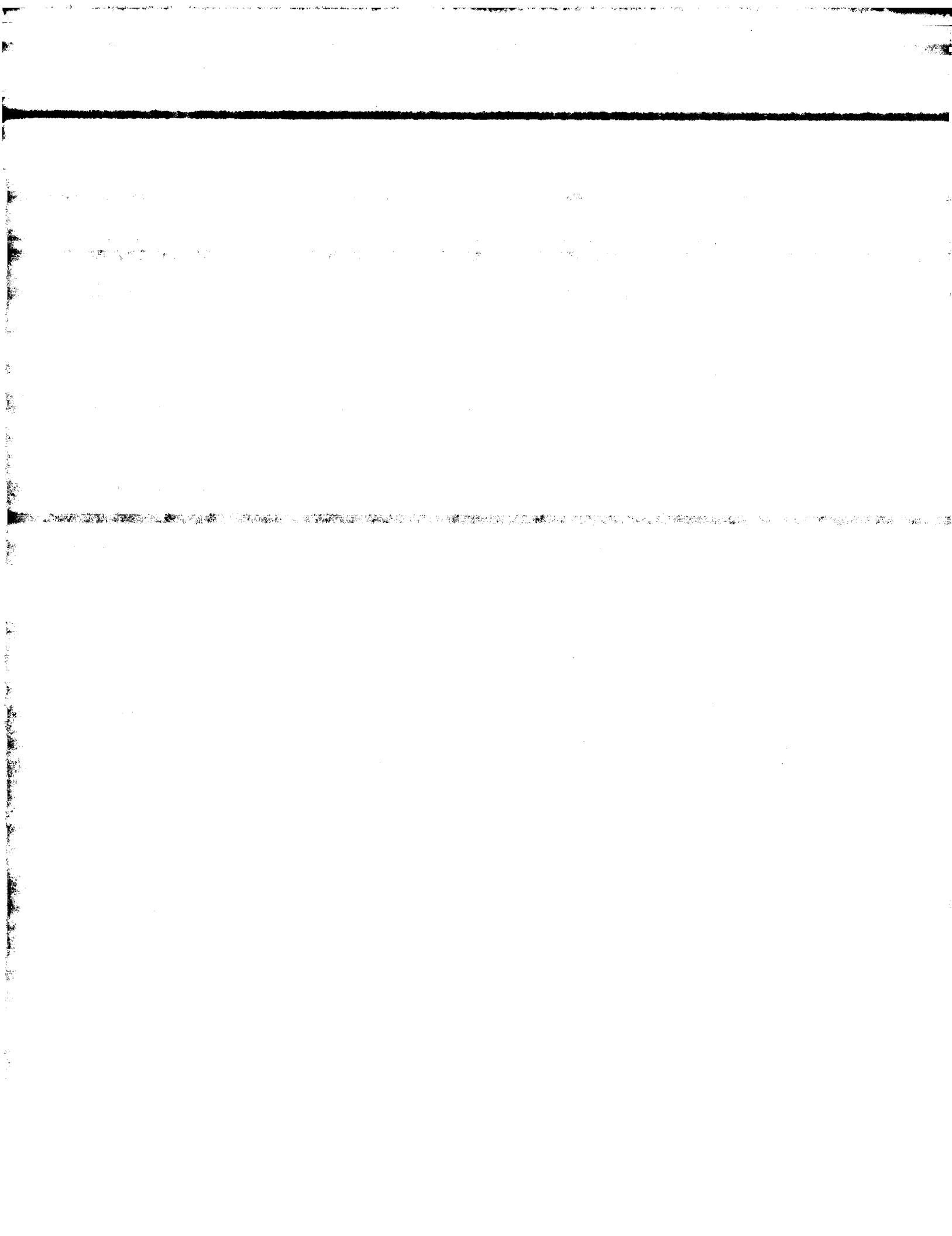
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FIG. 78.



$N=2'-O\text{-Me}$	$N=RIBO$
$U=2'-NH_2U$	$\textcircled{N}=TALO$

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C
SUBSTITUTE SHEET (RULE 26)



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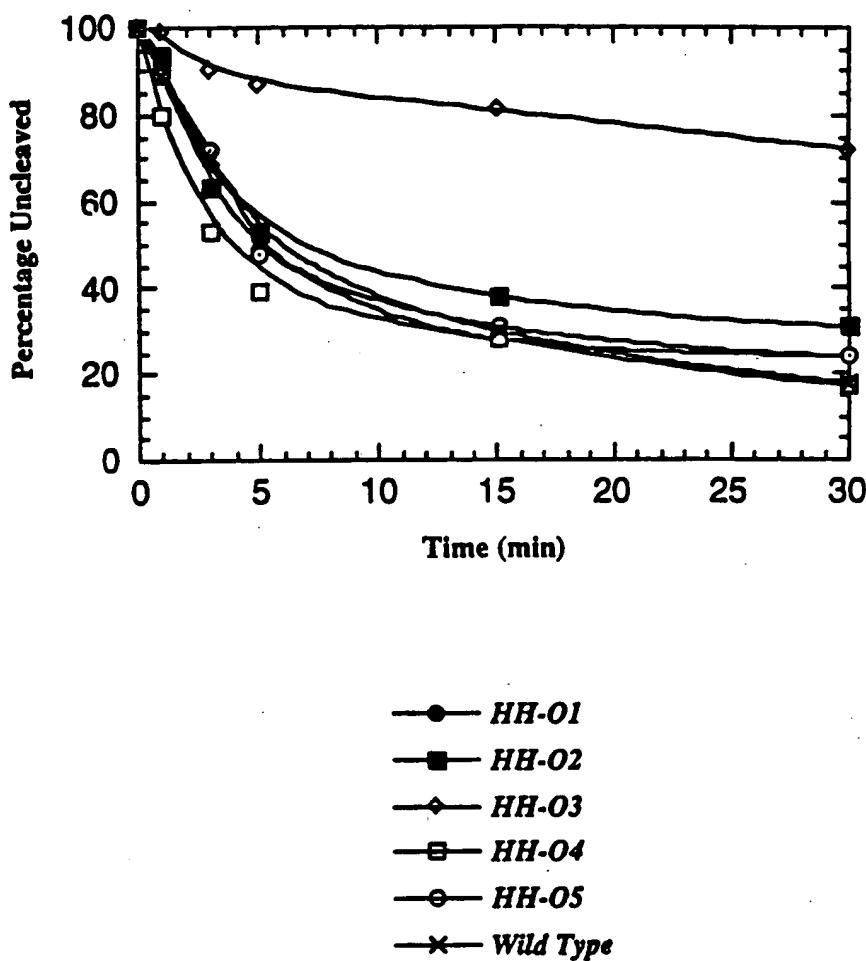
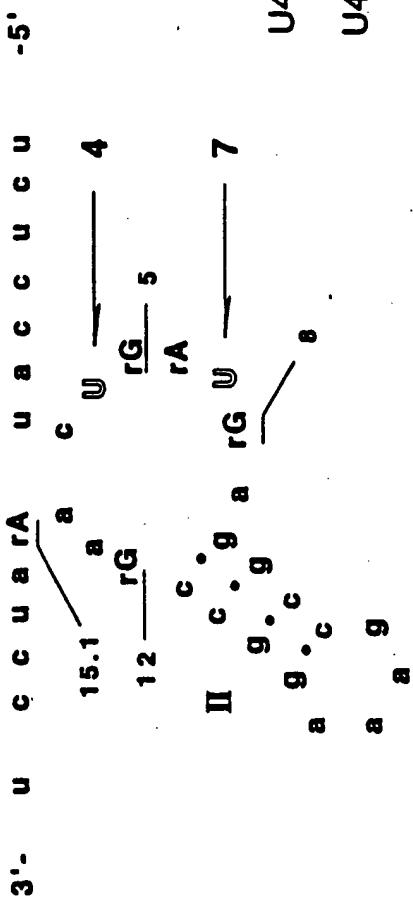


FIG. 79.

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Table 1 Entries

U4 & U7 = 2'-C-Allyl-U 12-14

U4 & U7 = 2'-F-ribo-U 9-11

U4 & U7 = 2'=CH₂-U 3-5

U4 & U7 = 2'=CF₂-U 6-8

U4 & U7 = 2'-dU 21-22

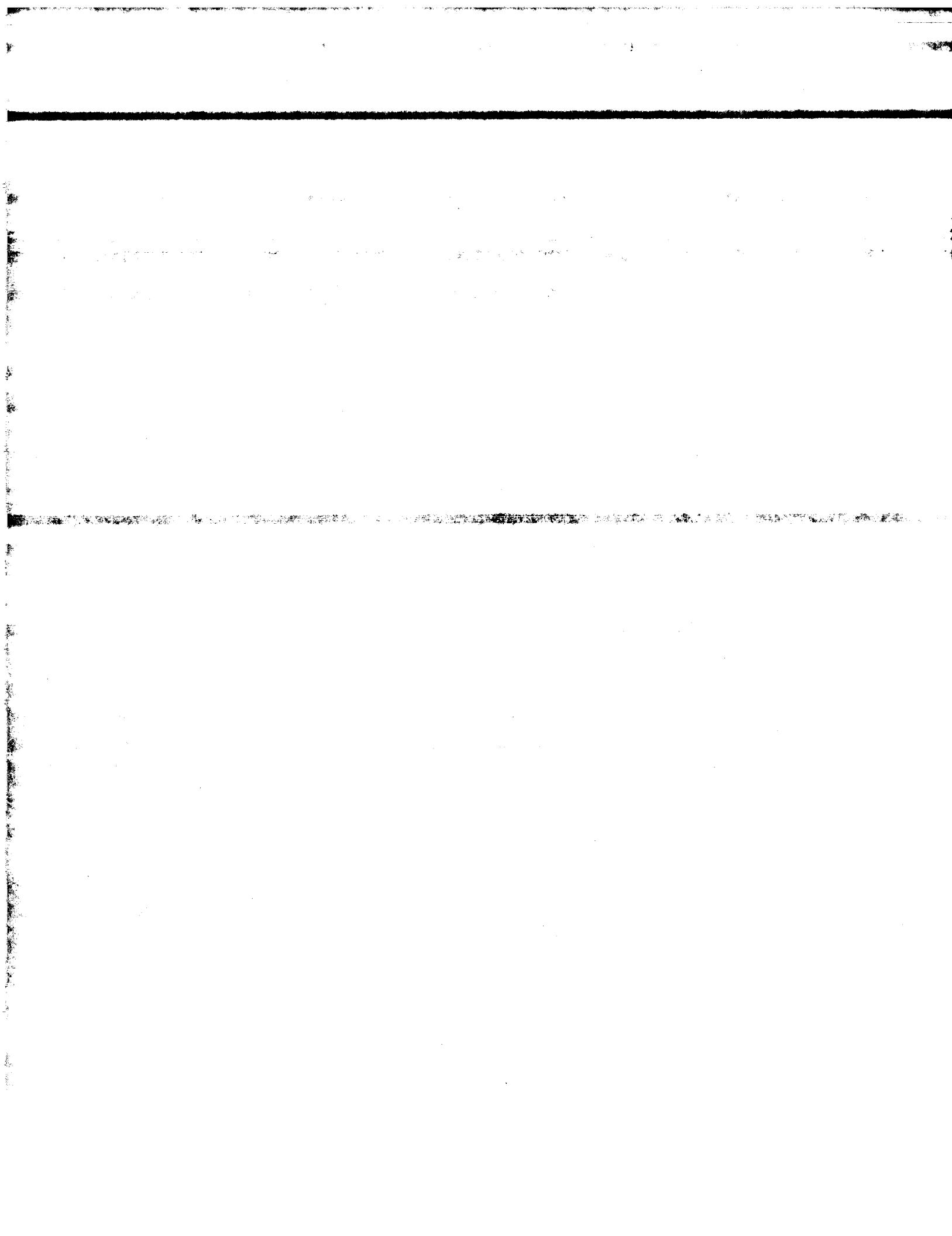
U4 & U7 = 2'-F-ara-U 15-17

U4 & U7 = 2'-NH₂-U 18-20

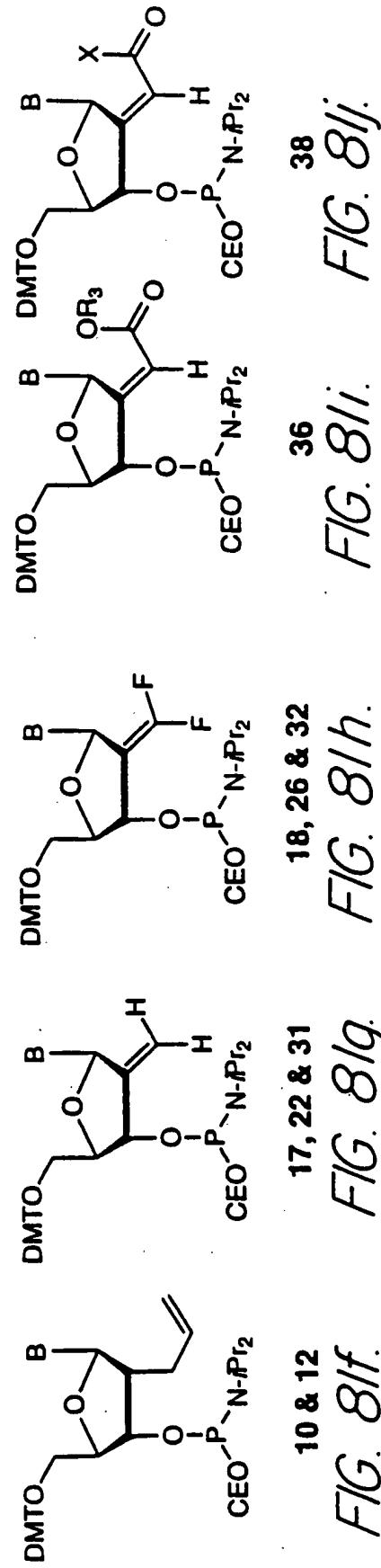
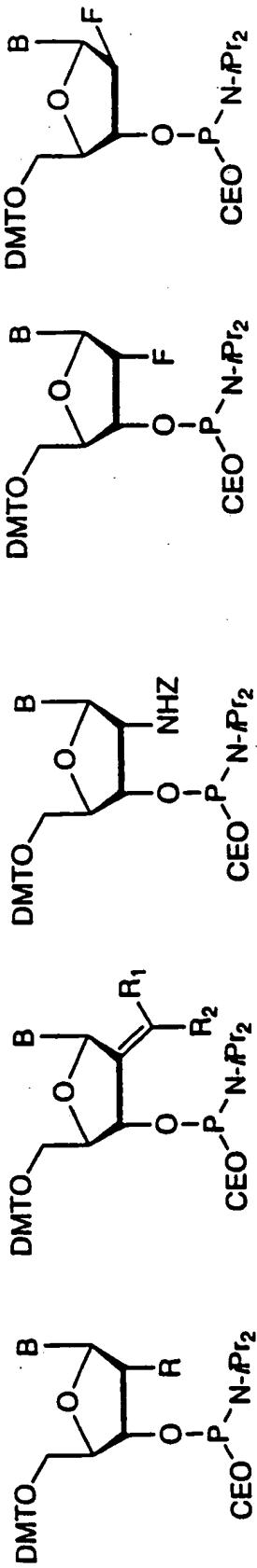
U4 & U7 = 2'-O-Me-ribo-U 2

Lower case = 2'-O-Me
ribonucleotide
rN = ribonucleotide

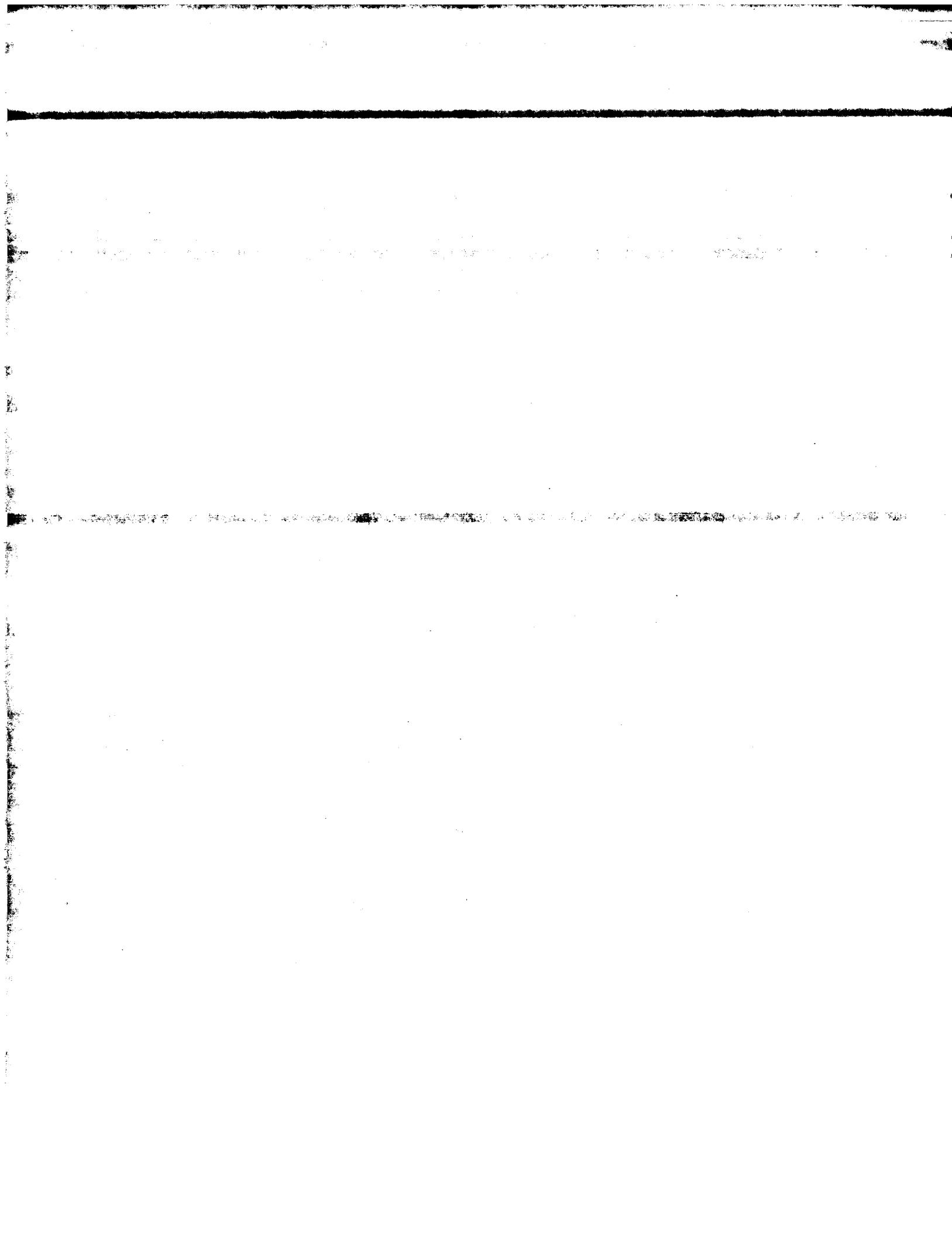
FIG. 80.



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B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.



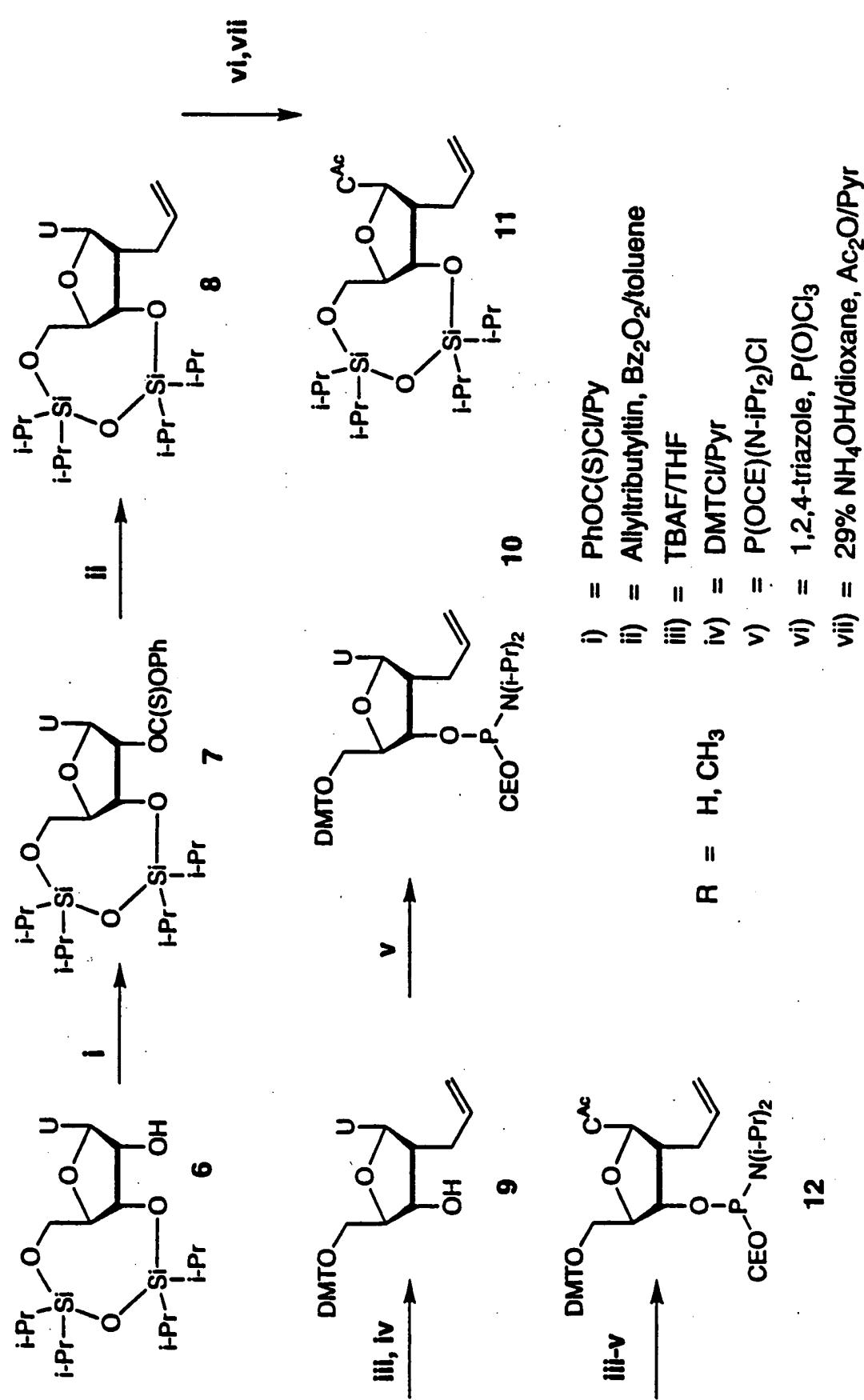
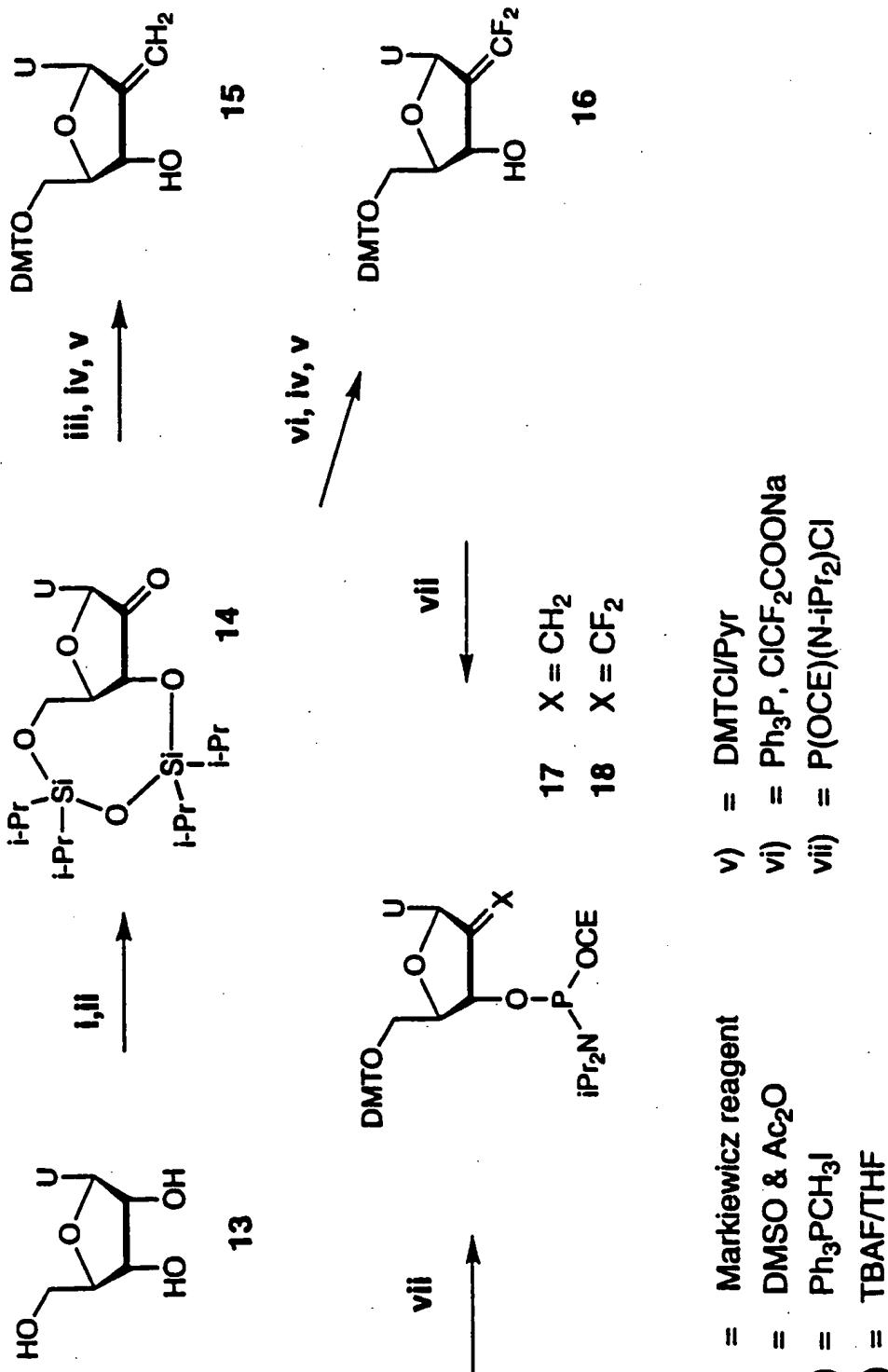


FIG. 82.

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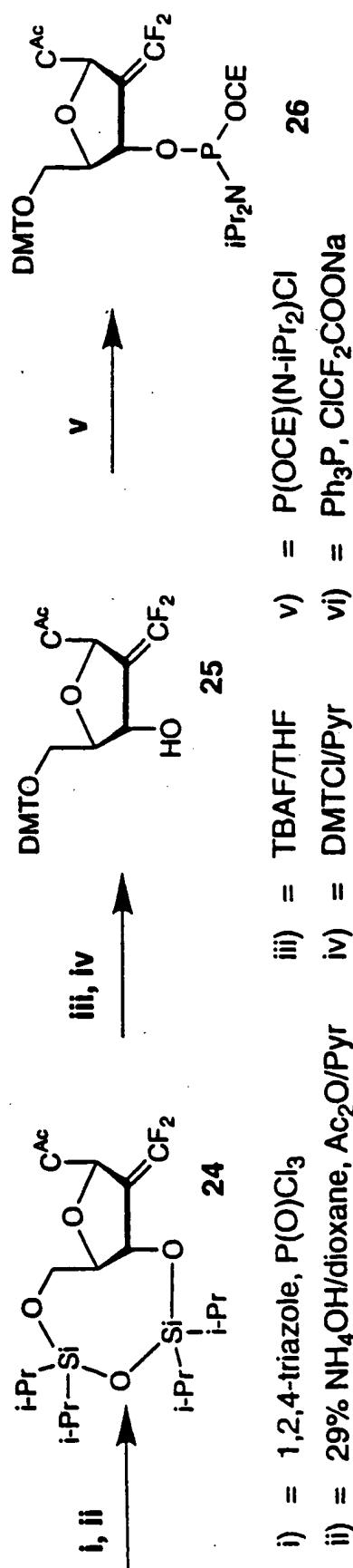
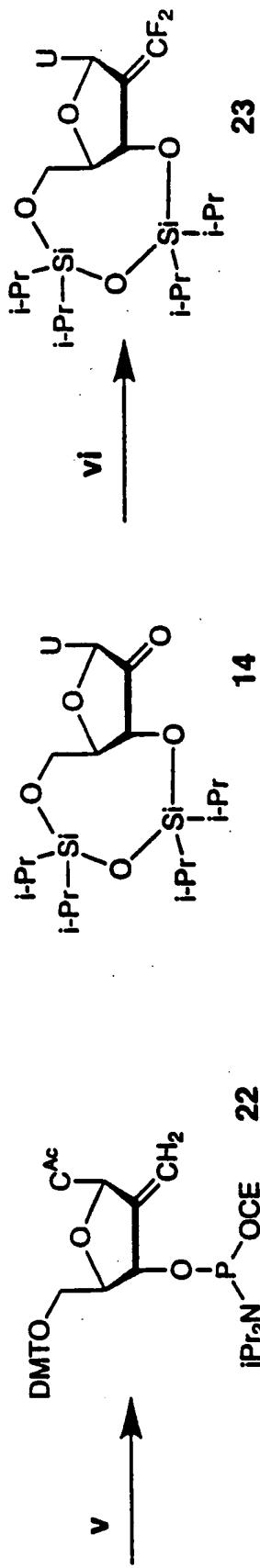
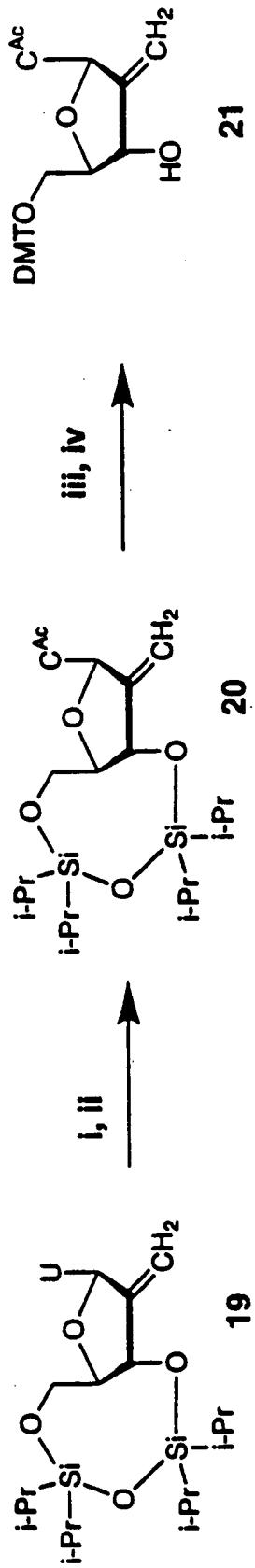
FIG. 83.

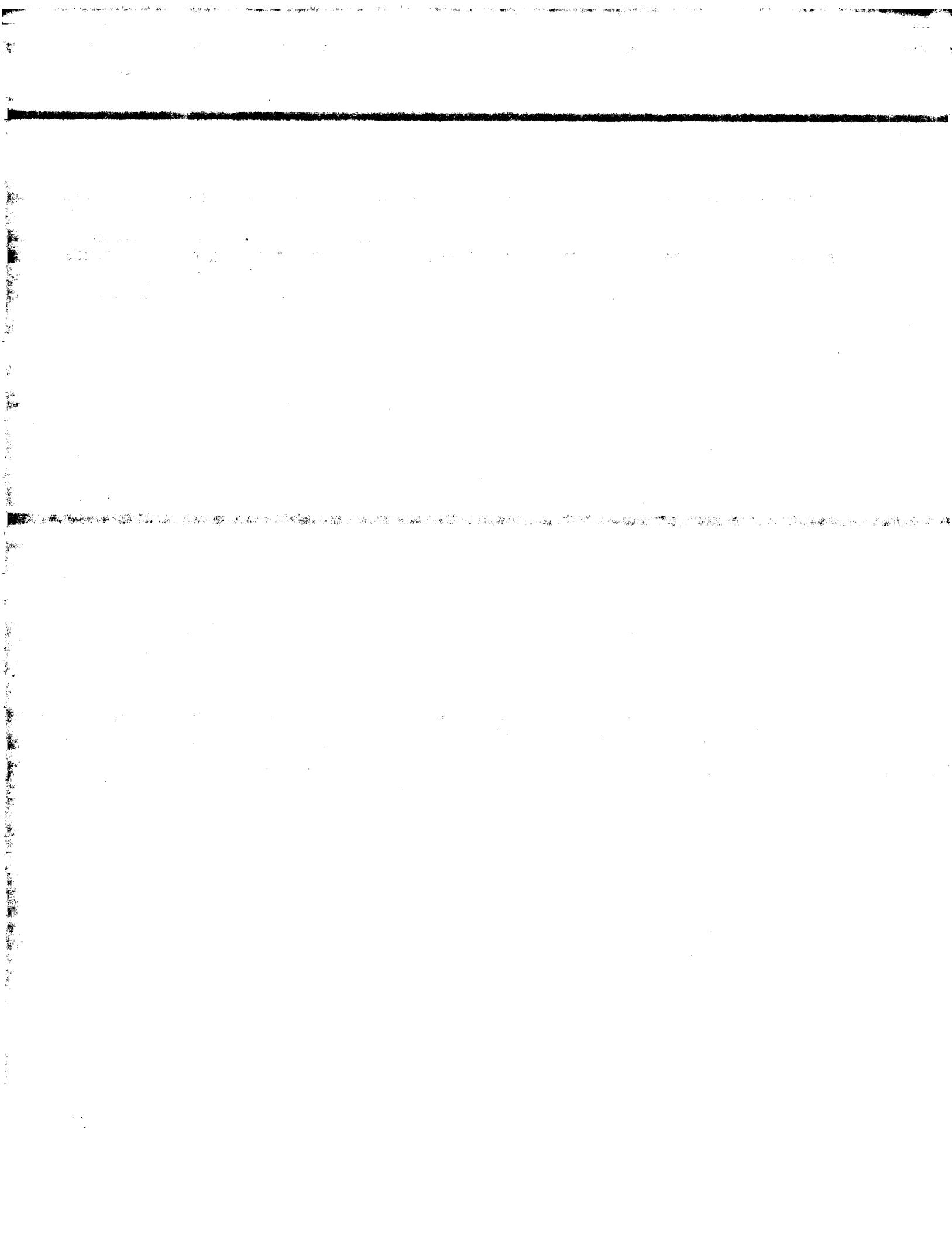


i)	=	Markiewicz reagent	v)	=	DMTCI/Pyr
ii)	=	DMSO & Ac ₂ O	vi)	=	Ph ₃ P, ClCF ₂ COONa
iii)	=	Ph ₃ PCH ₃ I	vii)	=	P(OCE)(N-iPr ₂)Cl
iv)	=	TBAF/THF			

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FIG. 84.





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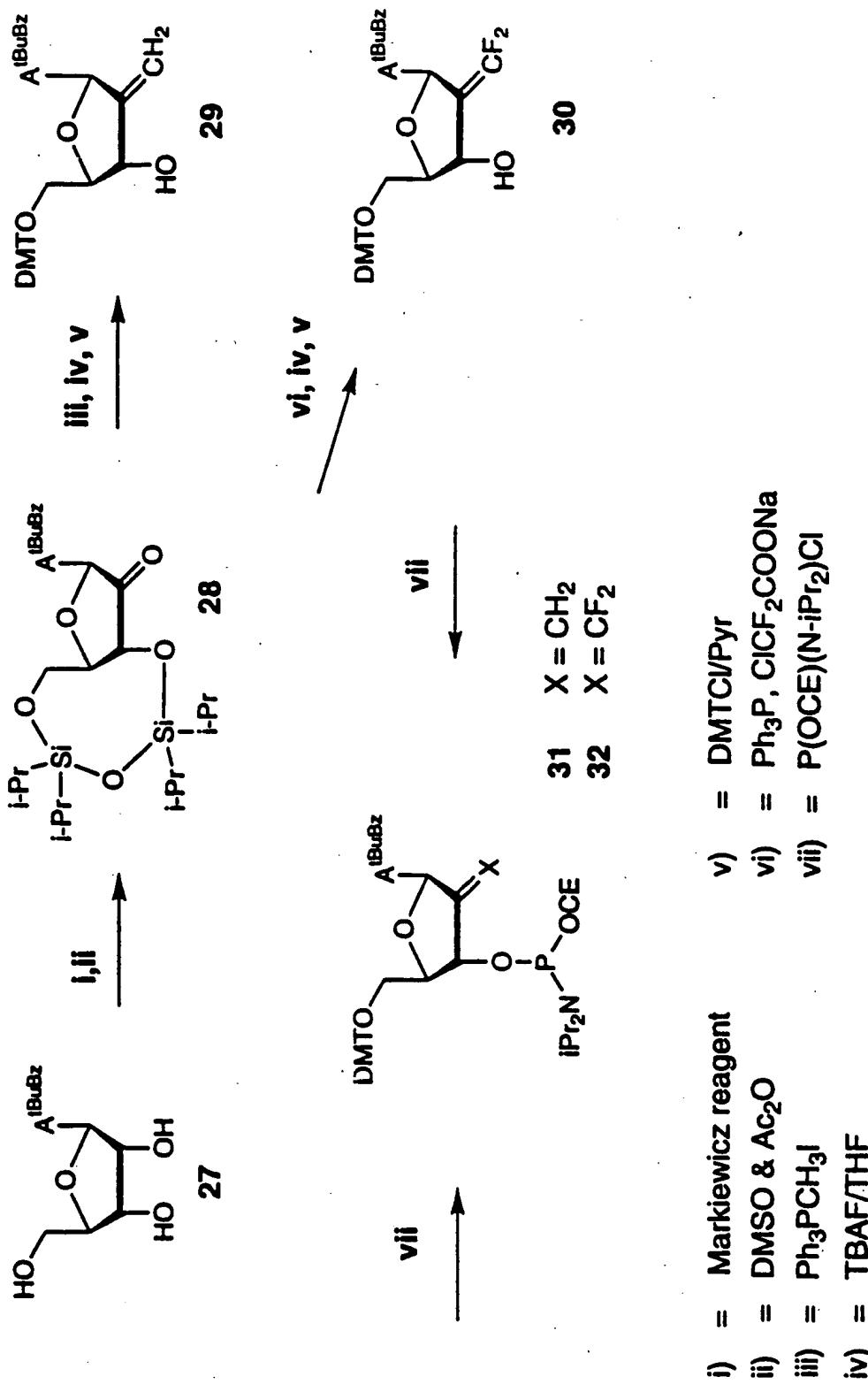
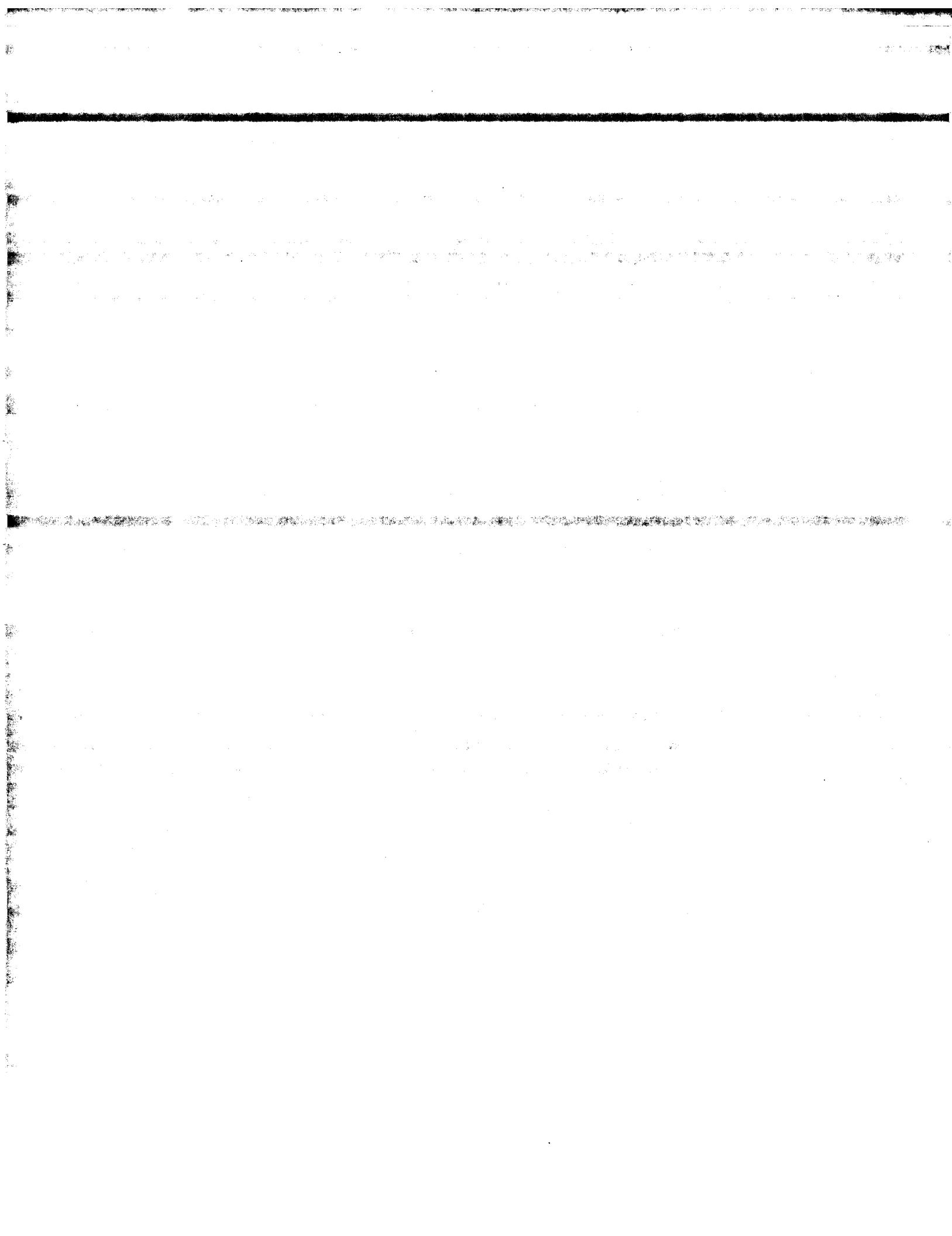
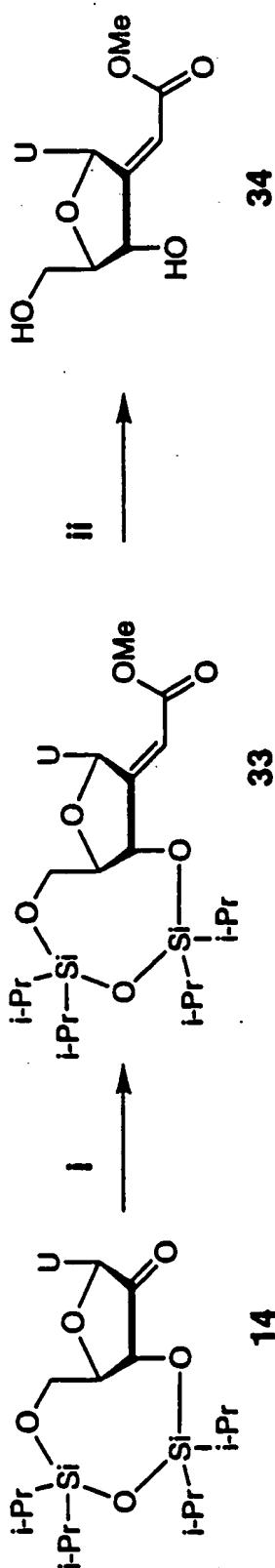


FIG. 85.



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- i) = $\text{Ph}_3\text{PC}=\text{CHC(O)OCH}_3\text{OAc}$
- ii) = $\text{NEt}_3\cdot 3\text{HF}$
- iii) = DMTCI/Pyr
- iv) = $\text{P(OCE)(N-iPr}_2\text{Cl)}$
- v) = MeOH/NaOH

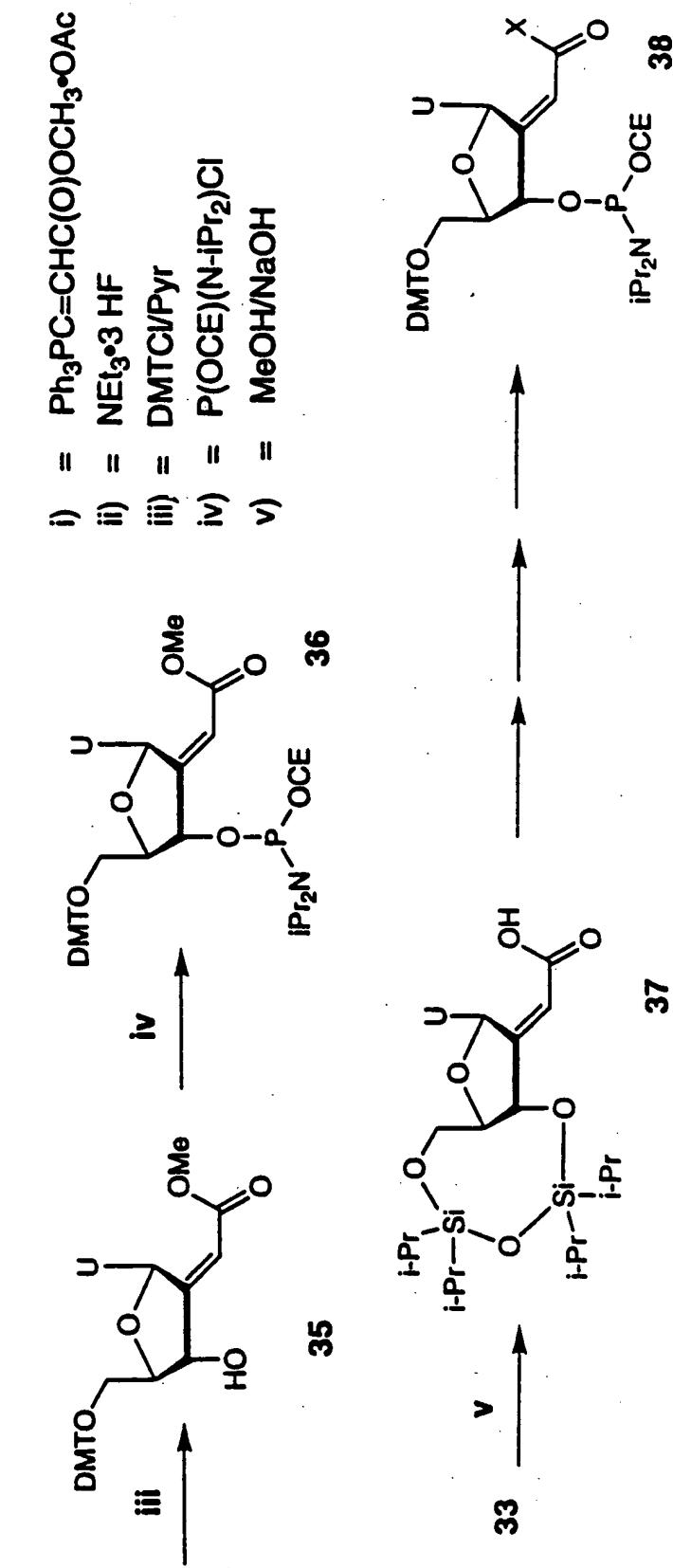
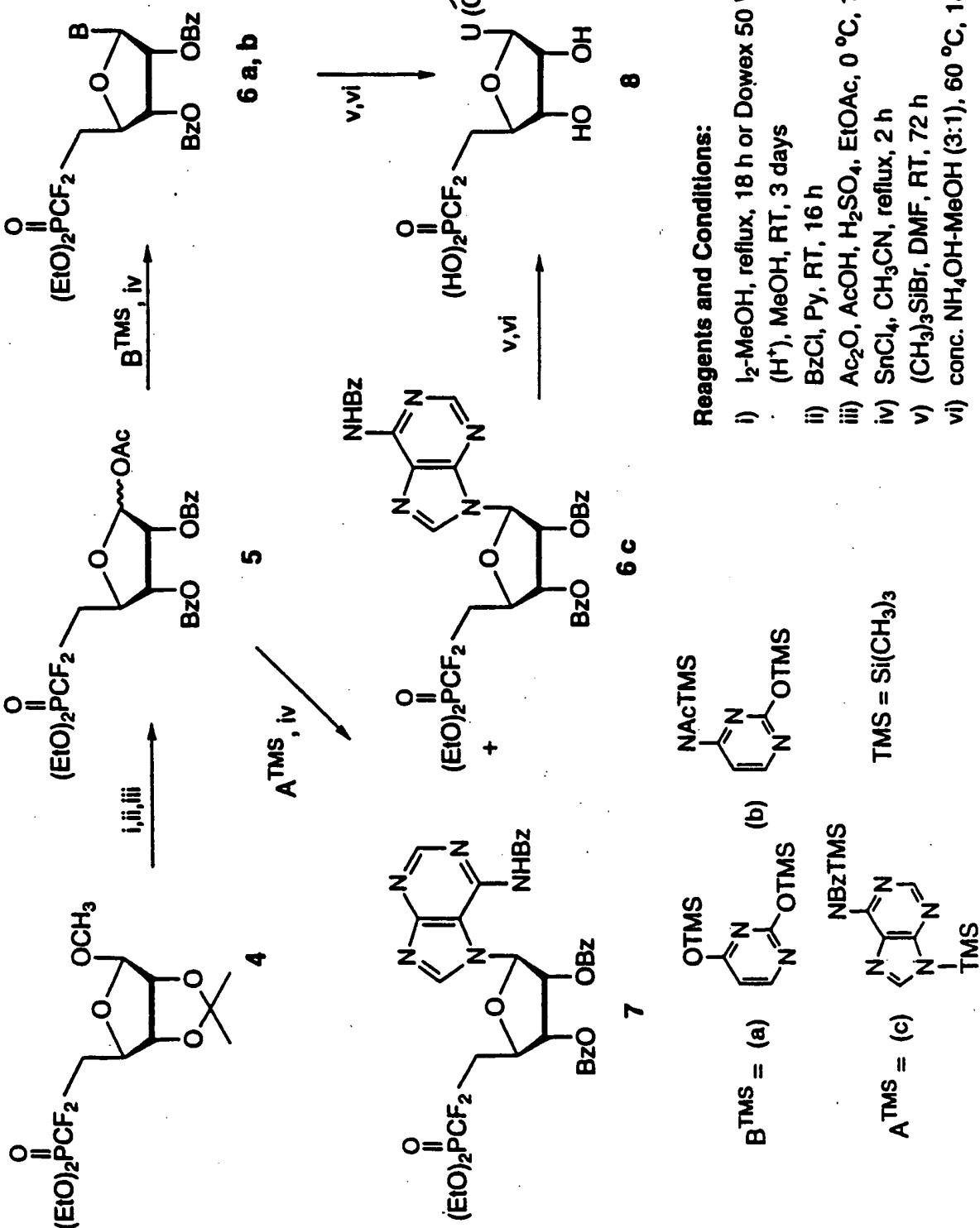
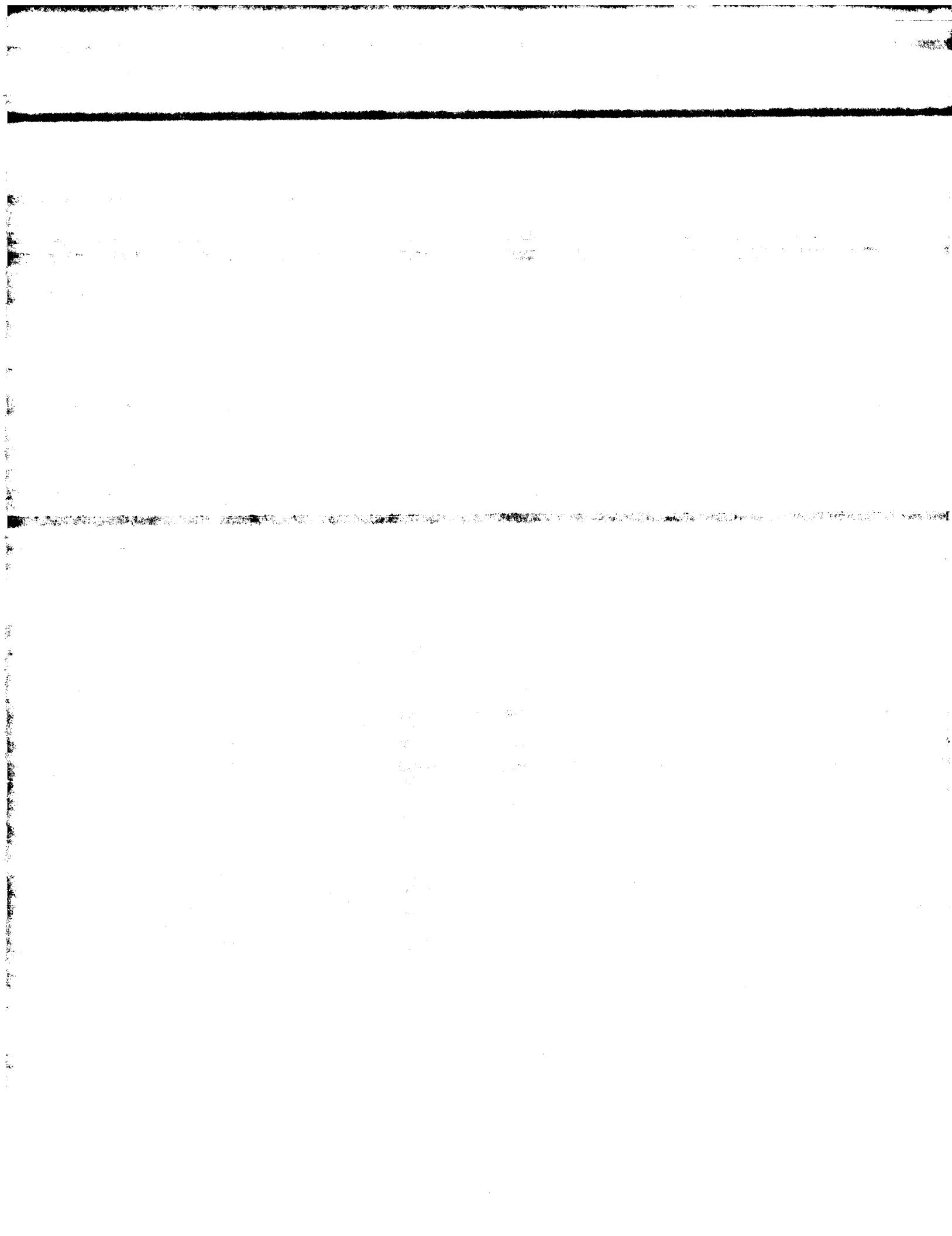


FIG. 86.

FIG. 87.

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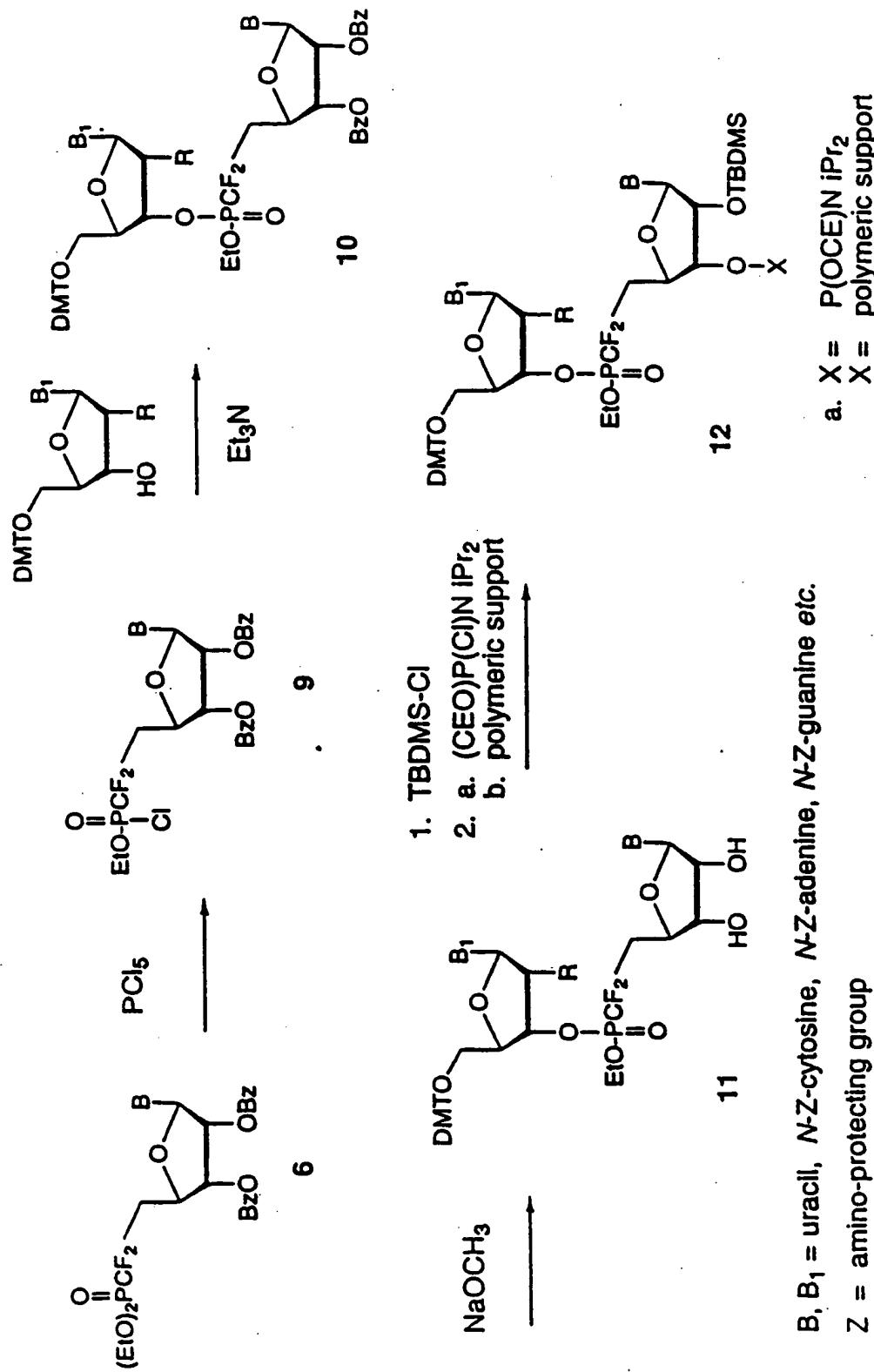
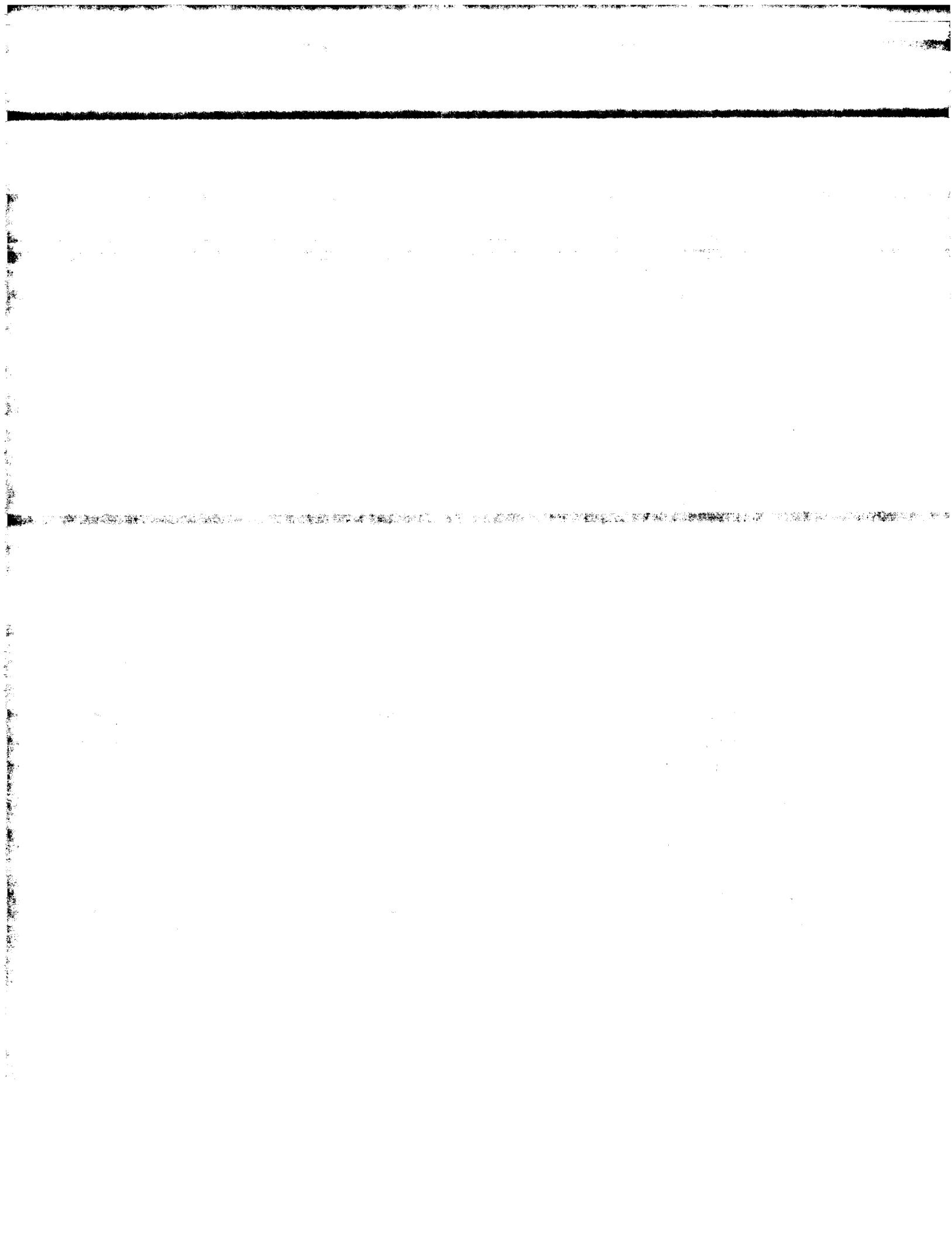


FIG. 88.



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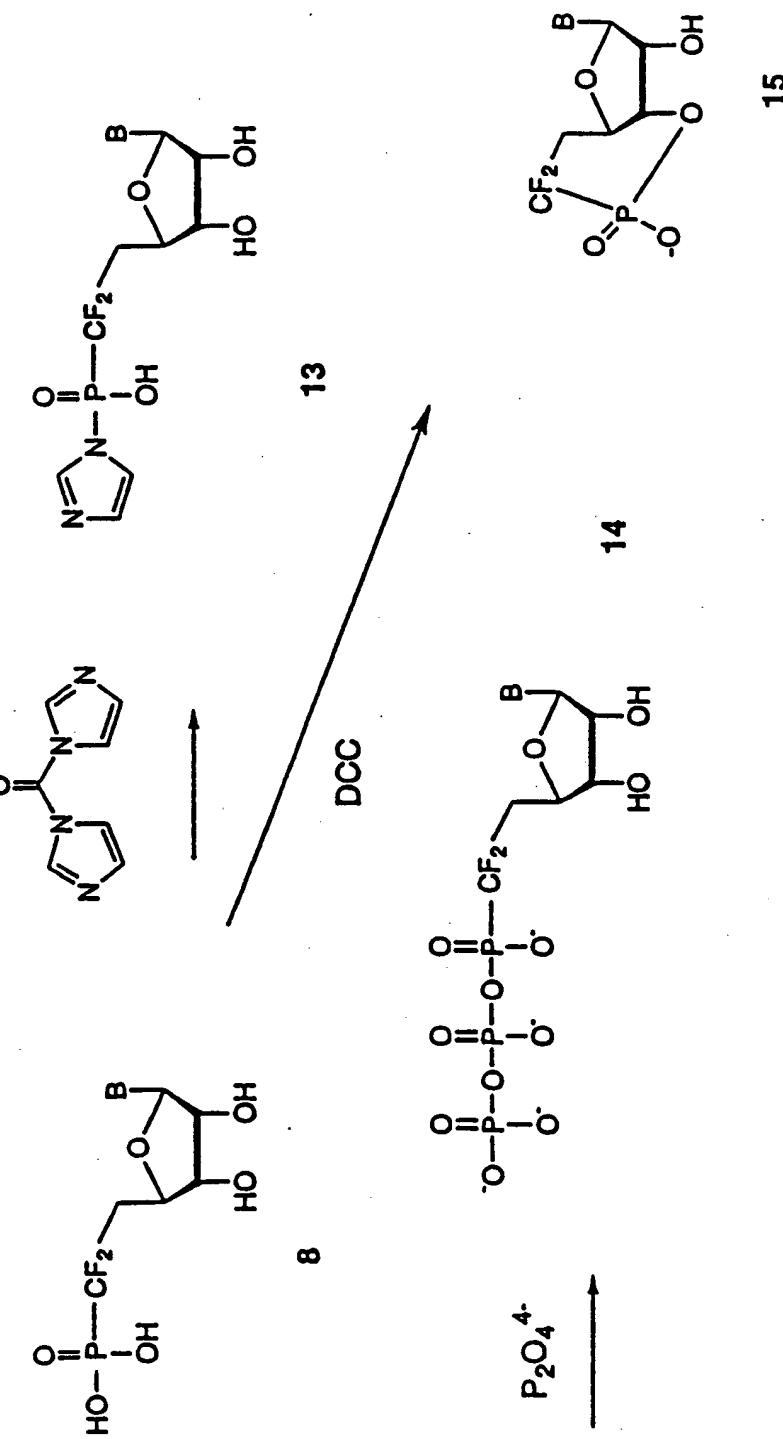
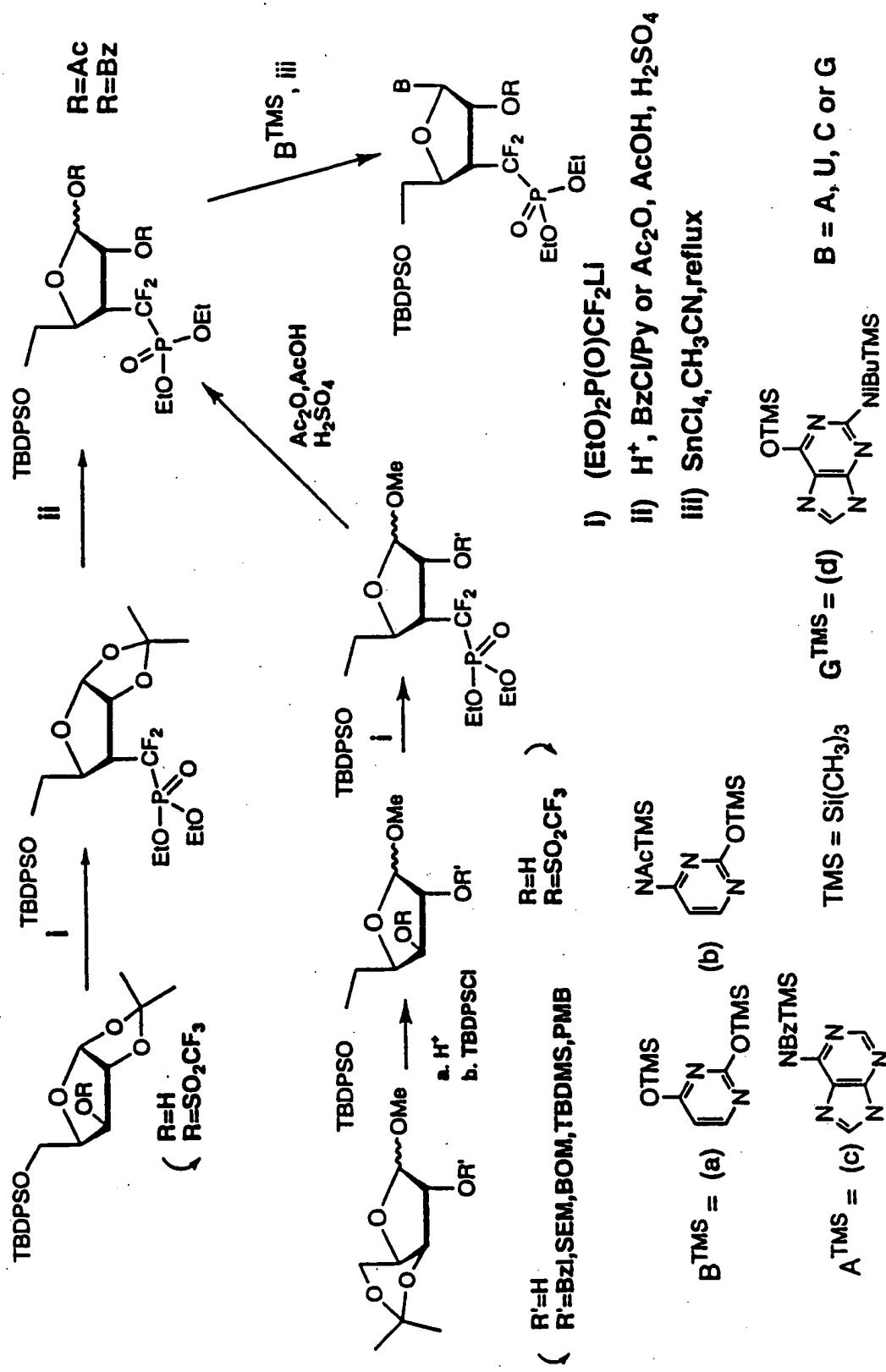
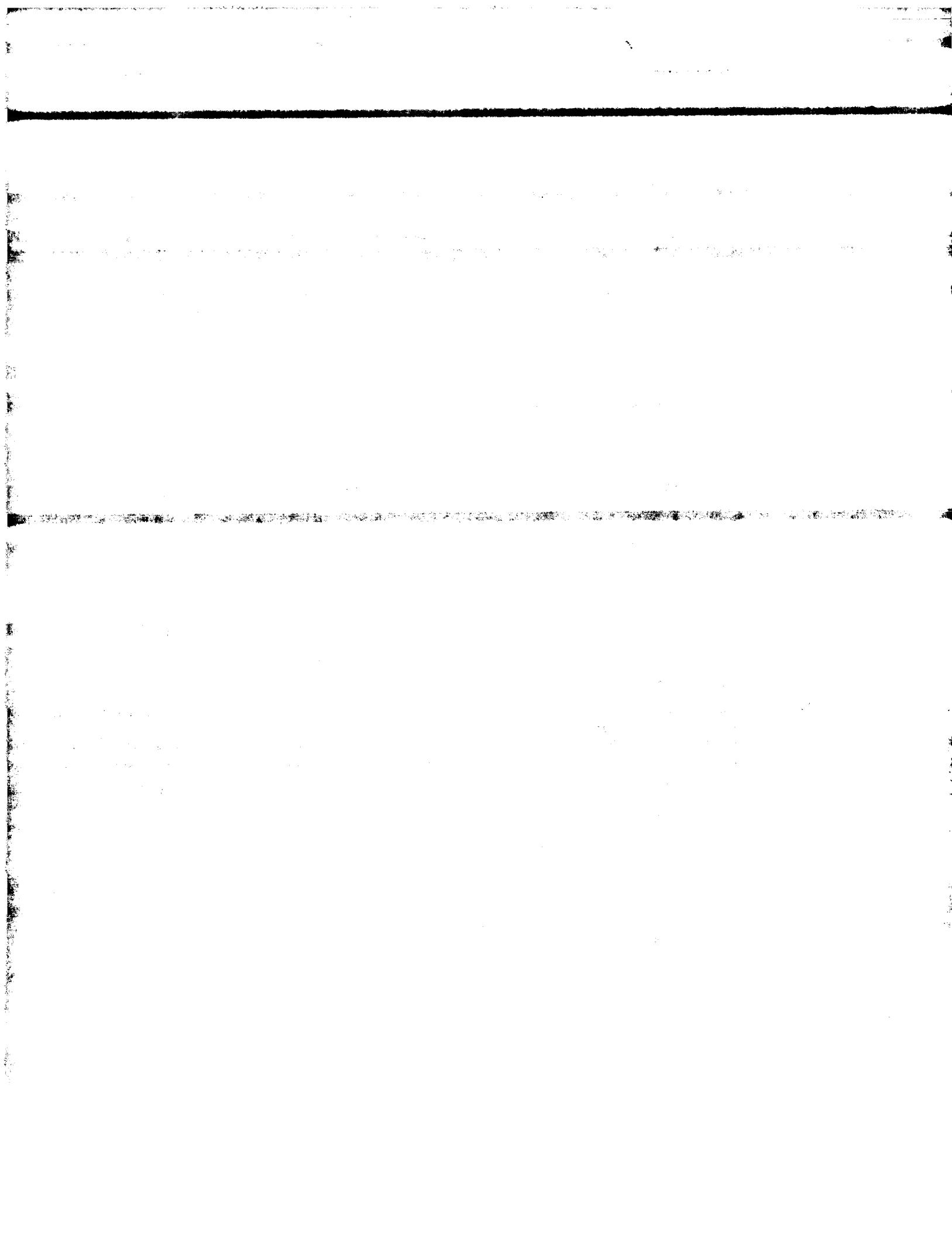


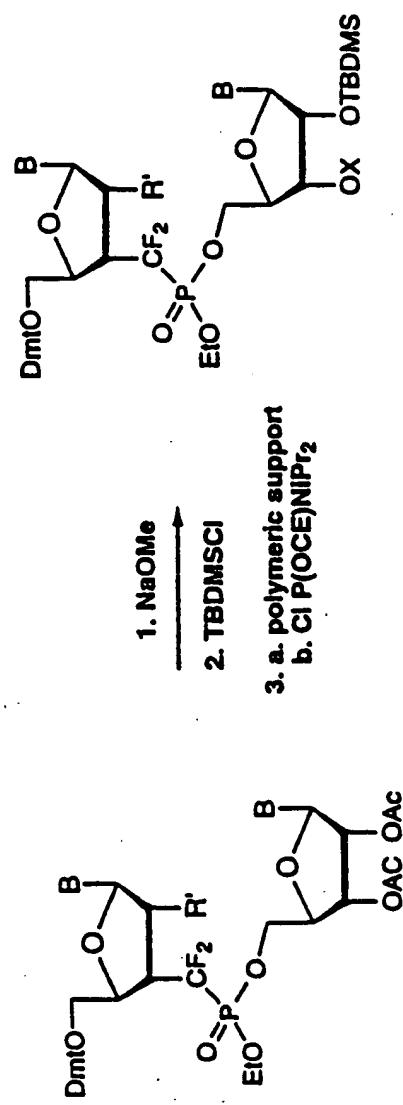
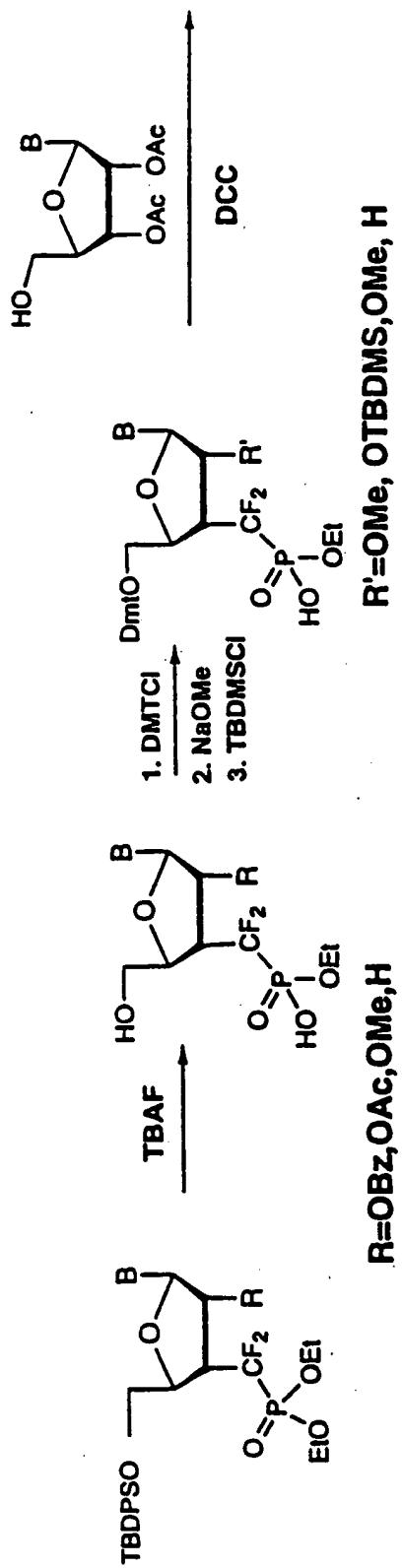
FIG. 89.

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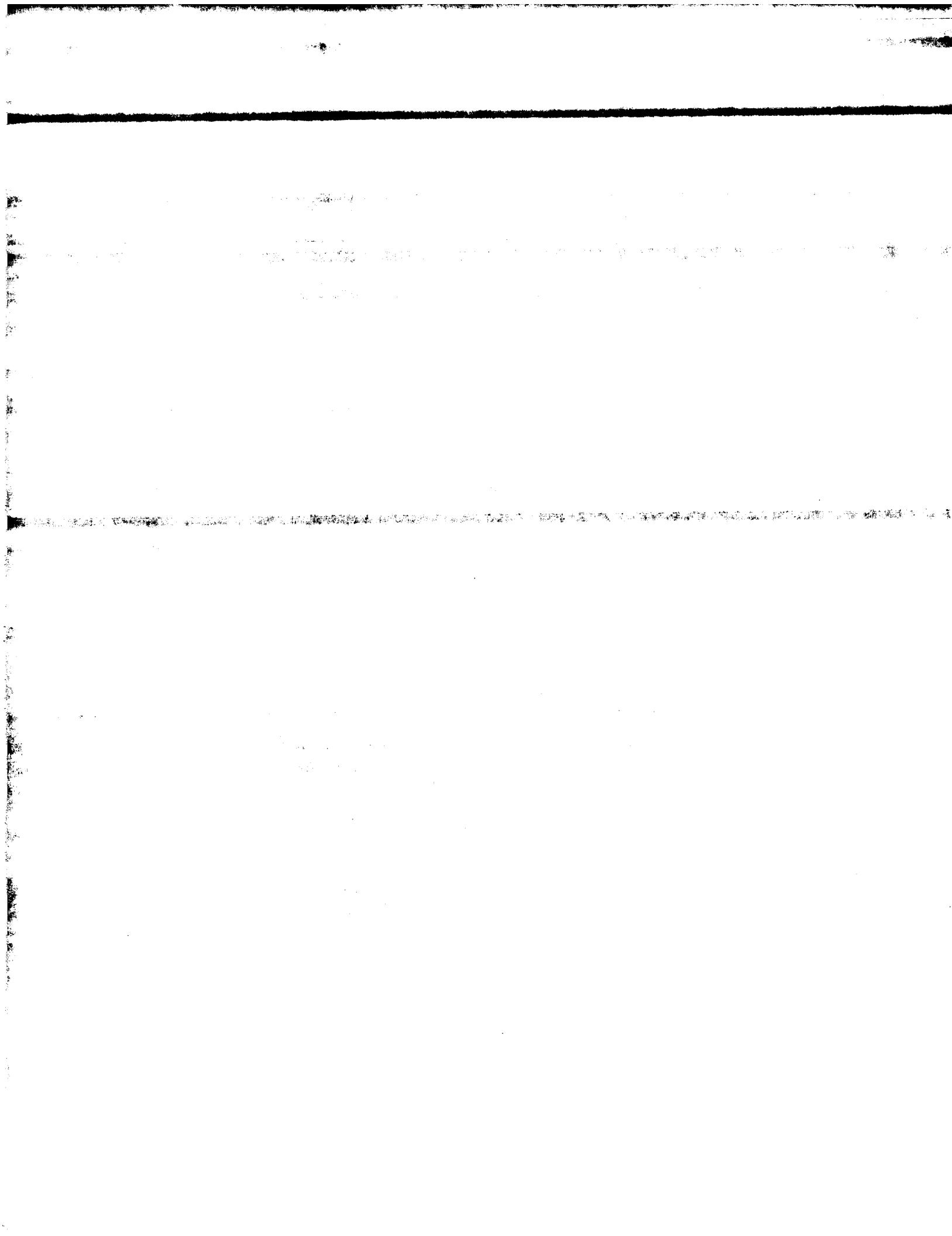


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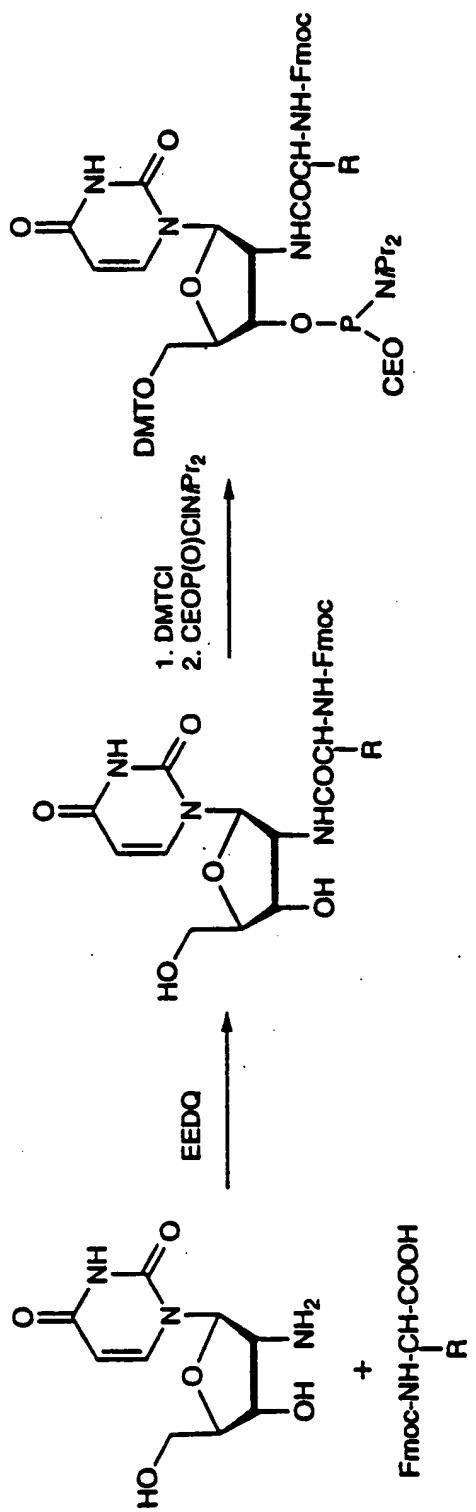


$\text{R}' = \text{OMe, OTBDMS, H}$
 $\text{X} = \text{polymeric support}$
 $\text{X} = \text{P(OCE)NIPr}_2$

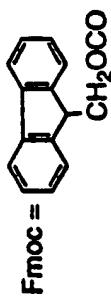
FIG. 9.



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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

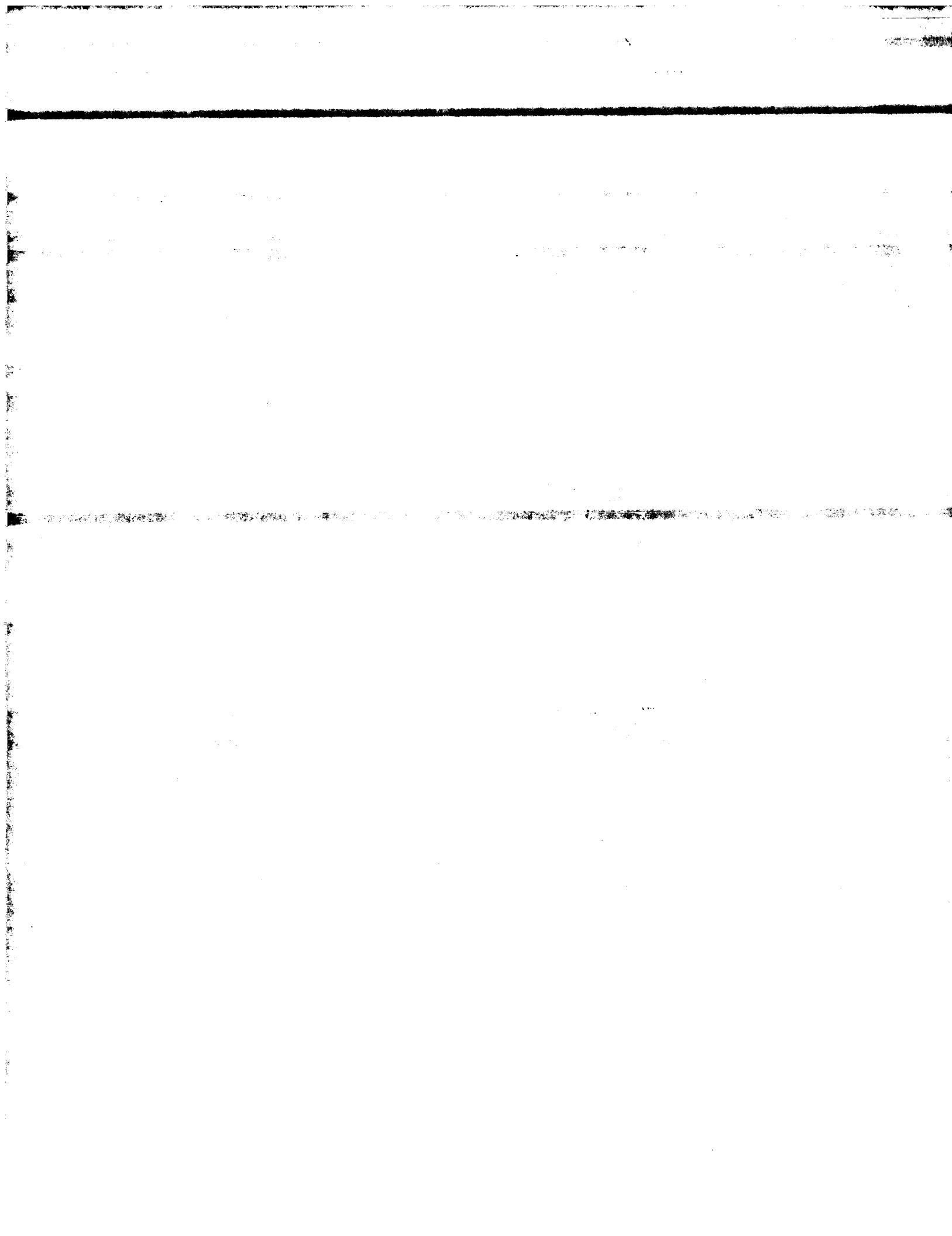


R = CH₃, CH₂-, (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBz (lys)

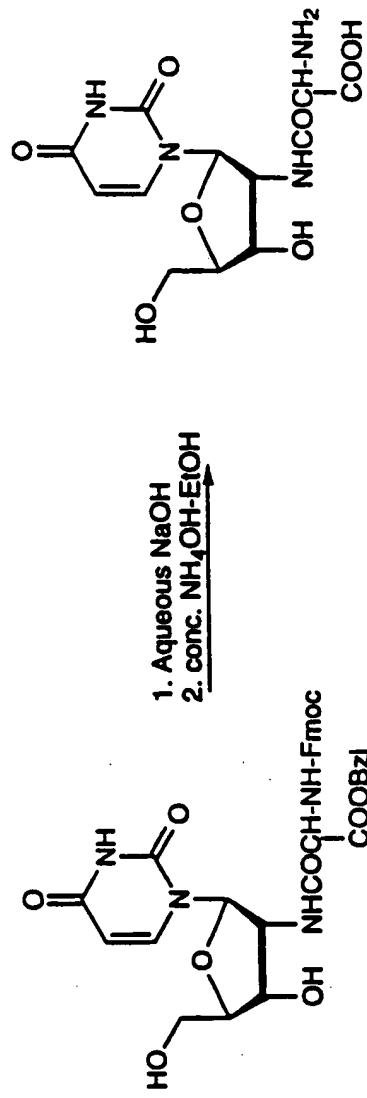
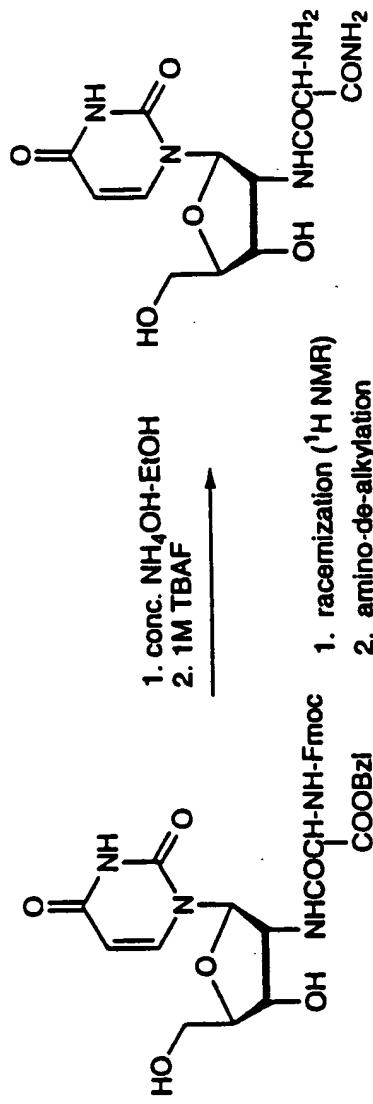
CBZ =

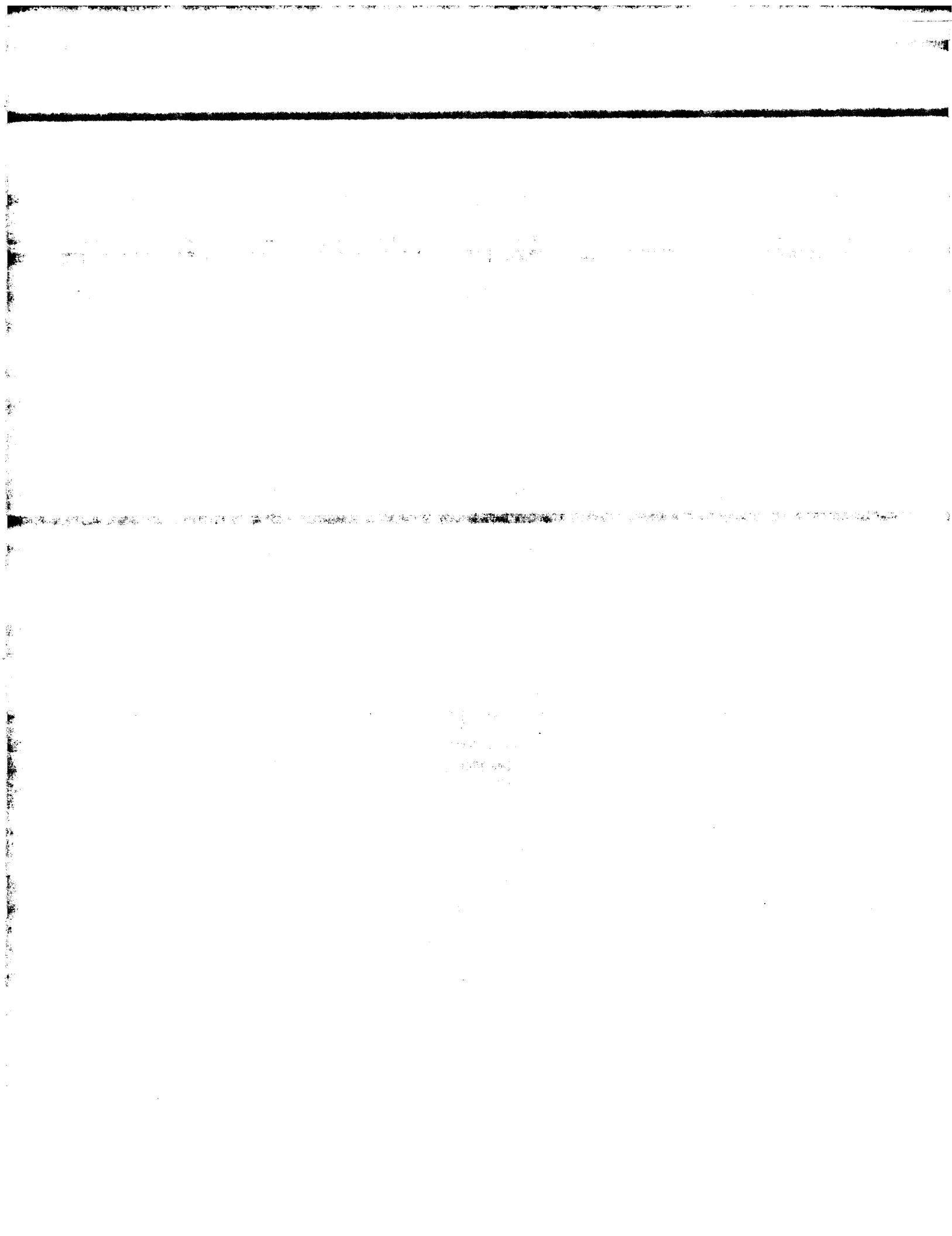
BzI =

FIG. 92.



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*FIG. 93a.***A***FIG. 93b.***B**



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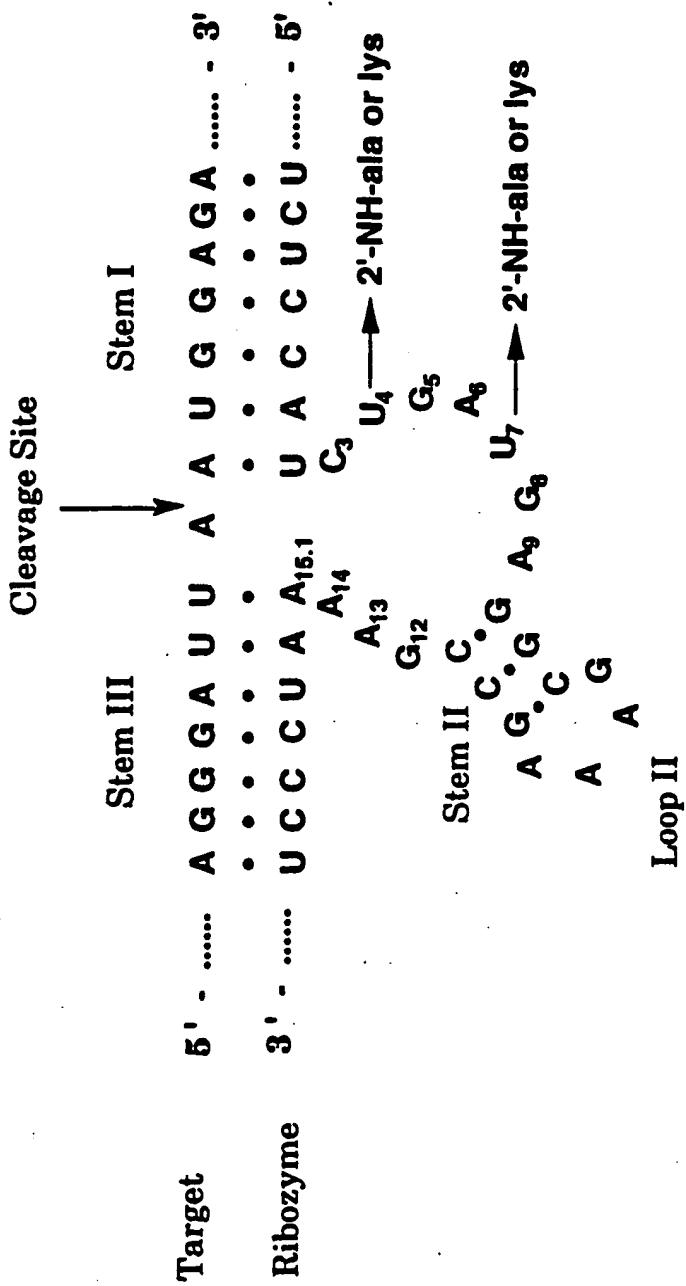
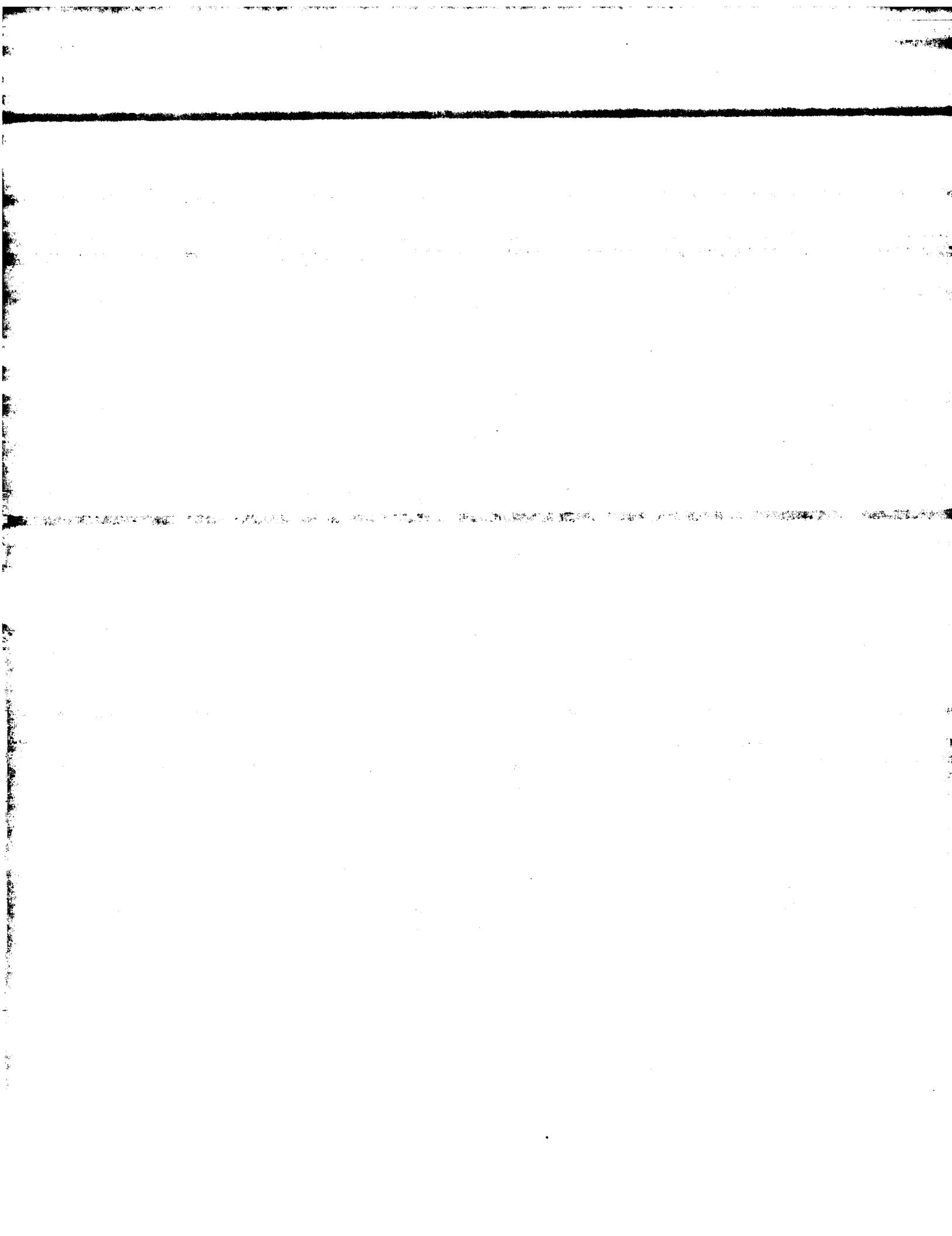
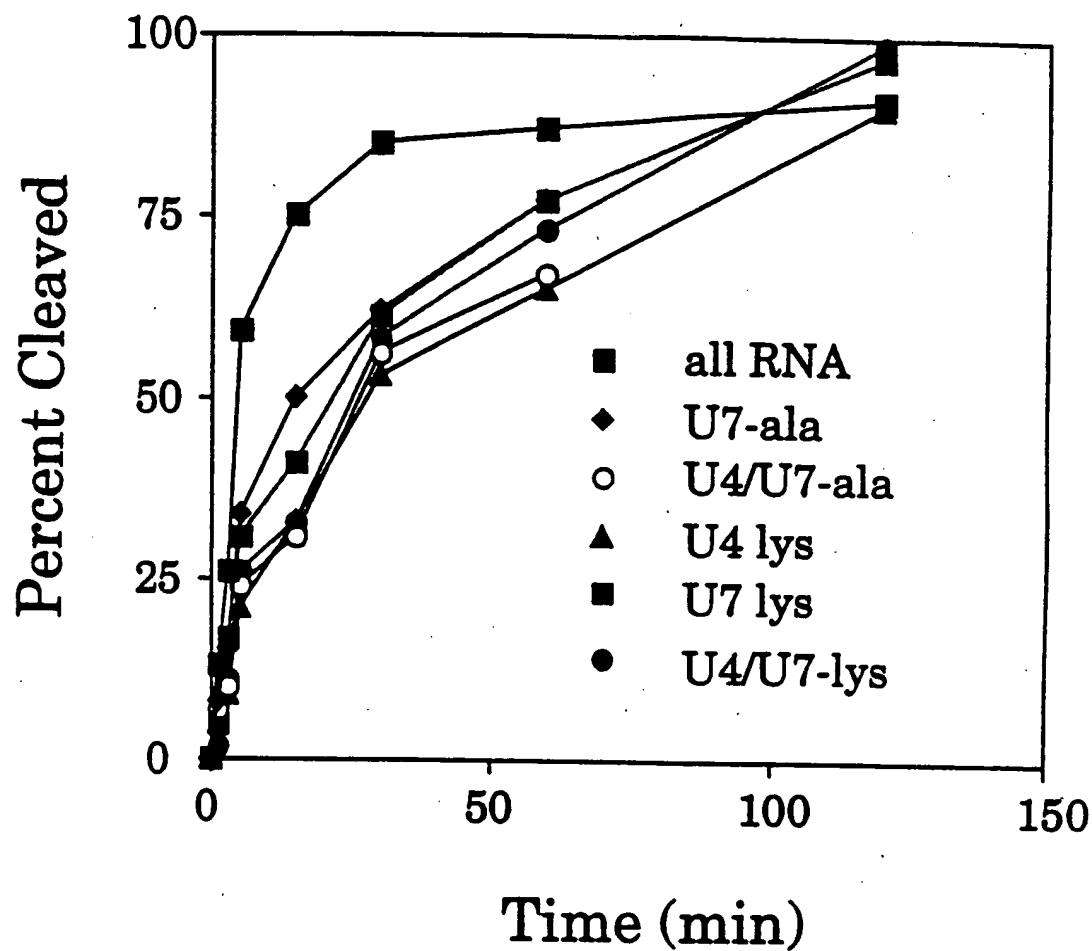


FIG. 94

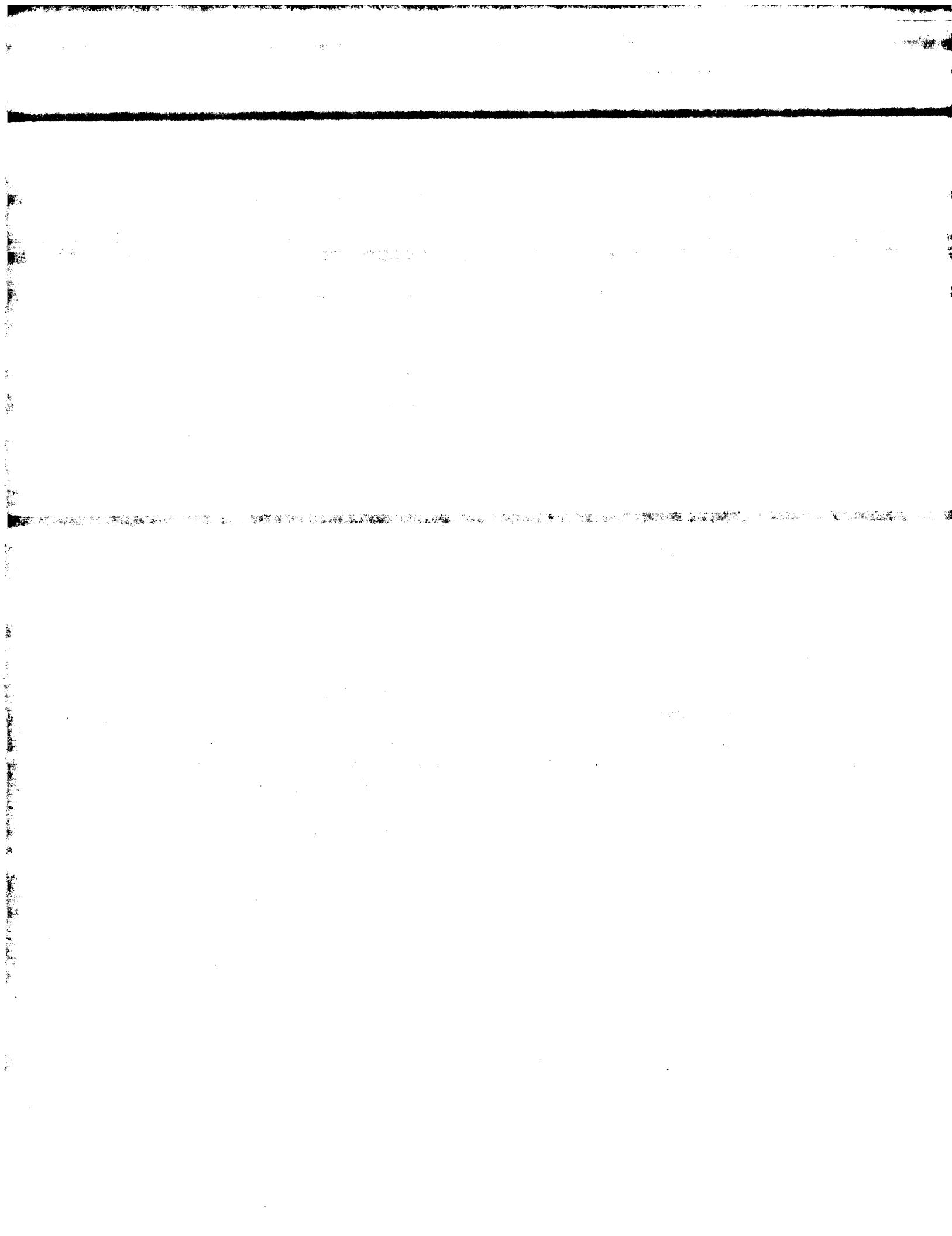


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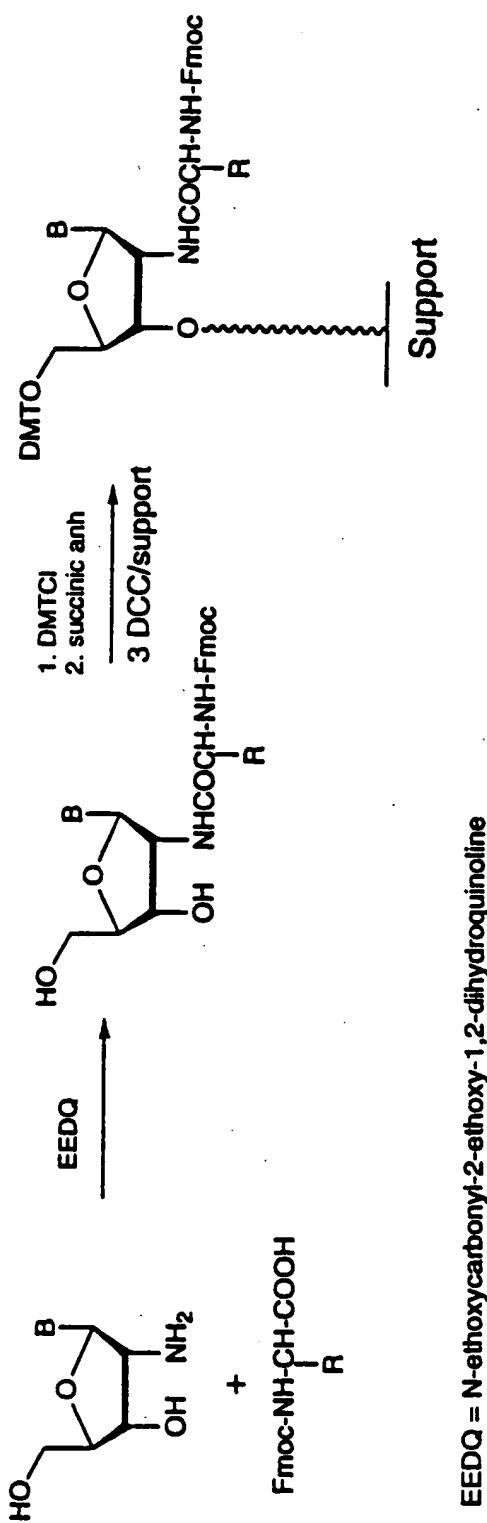


[Ribozyme] = 40 nM [Substrate] = ~1nM

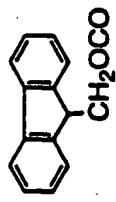
FIG. 95.



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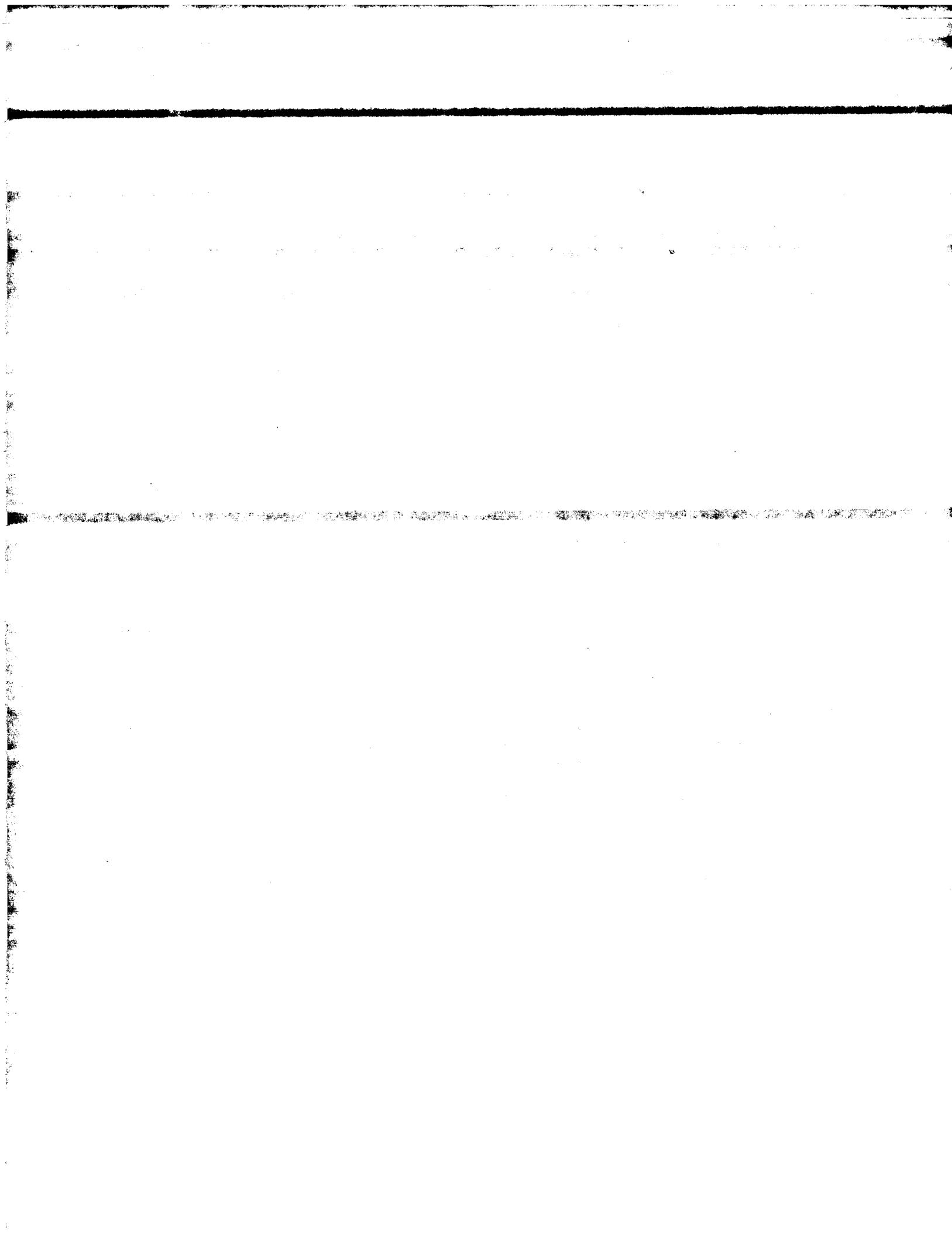


EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



$$\text{BzI} = \text{C}_6\text{H}_5\text{CH}_2$$

FIG. 96.



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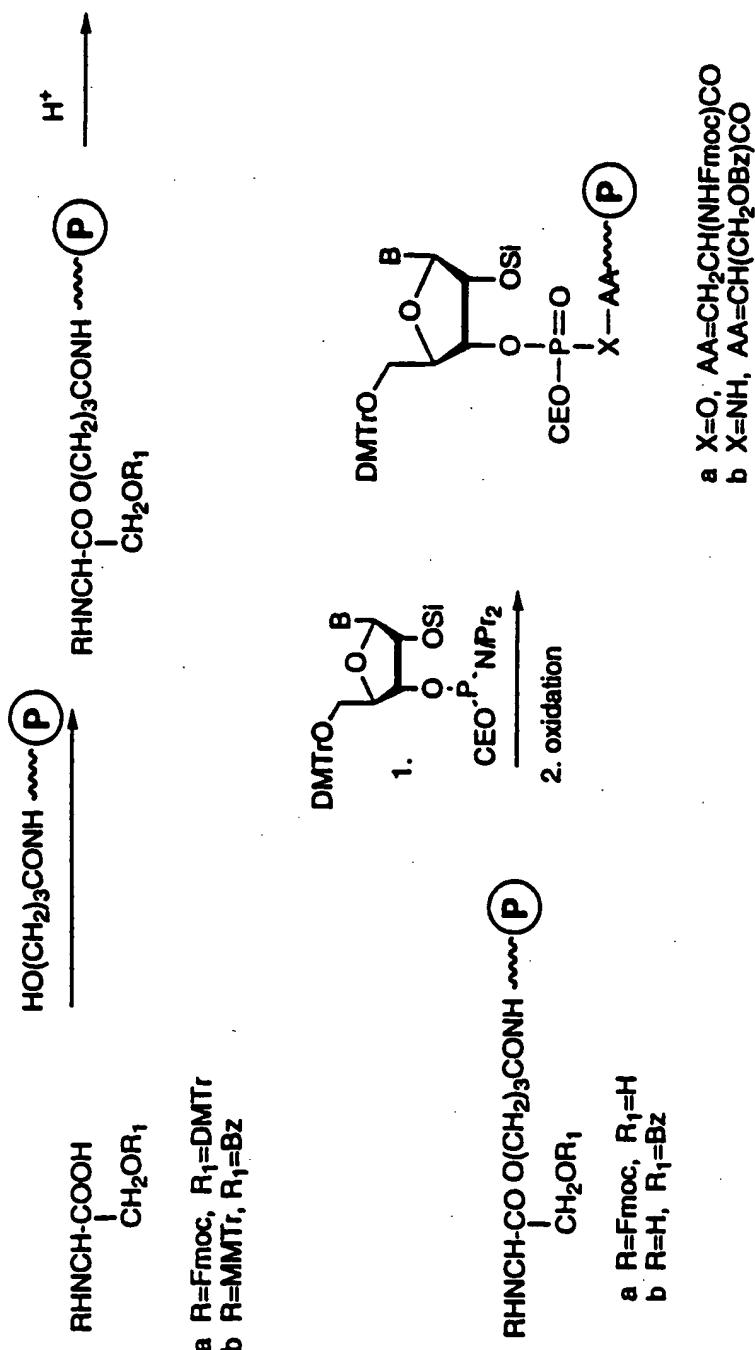
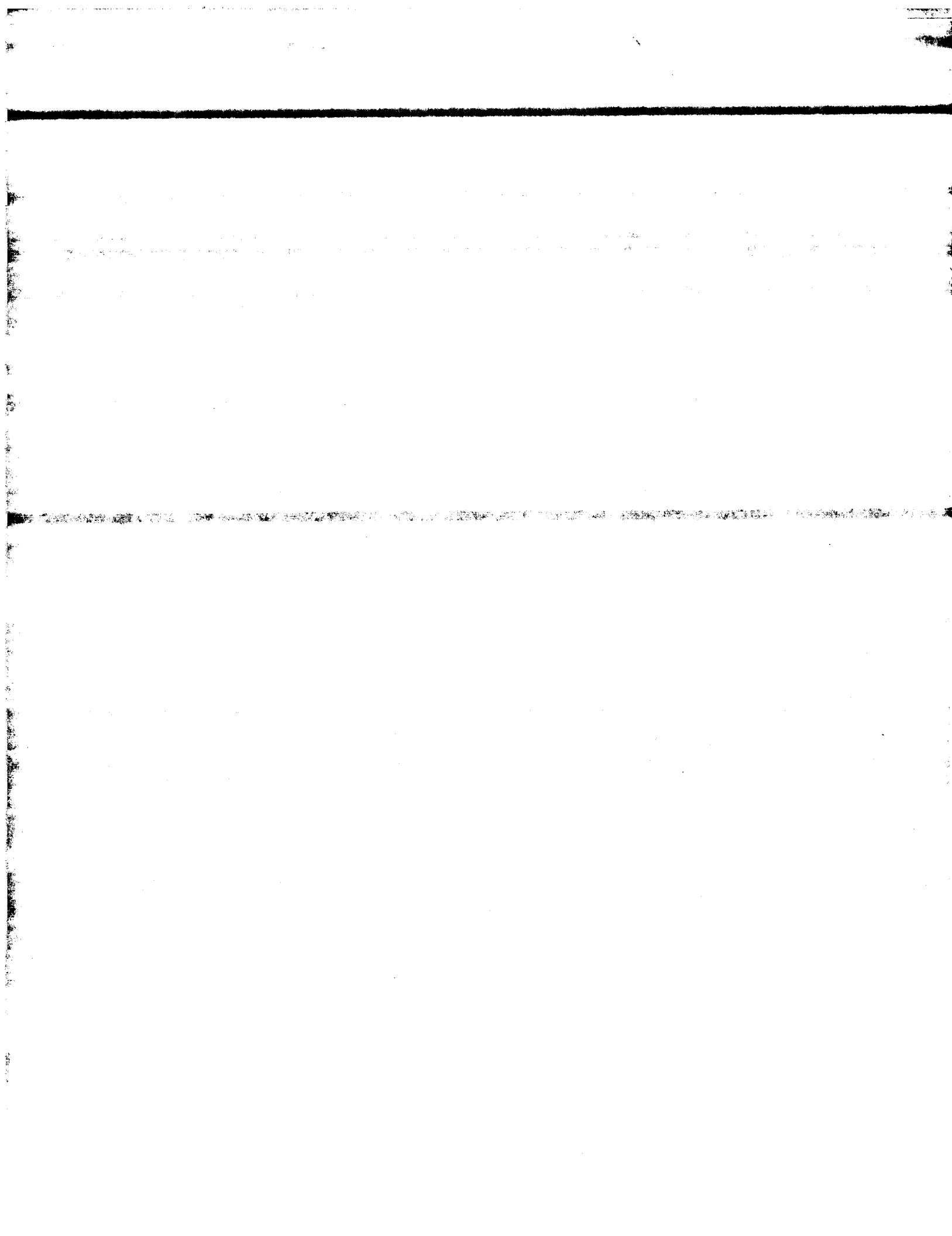
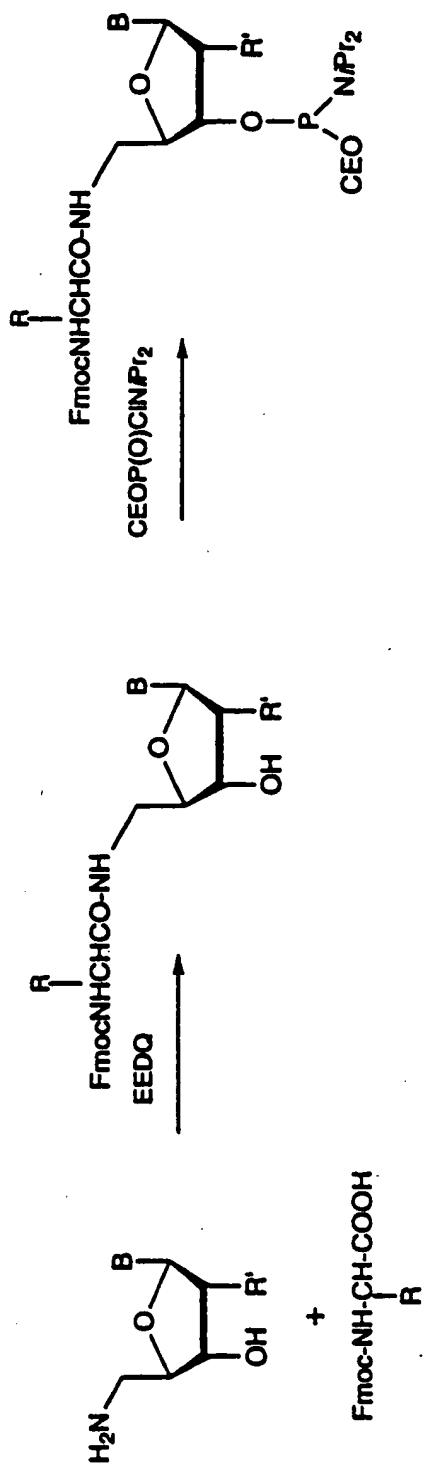
B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{bz}, mod. base, H

FIG. 97.



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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

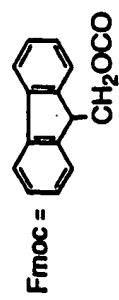


FIG. 98.

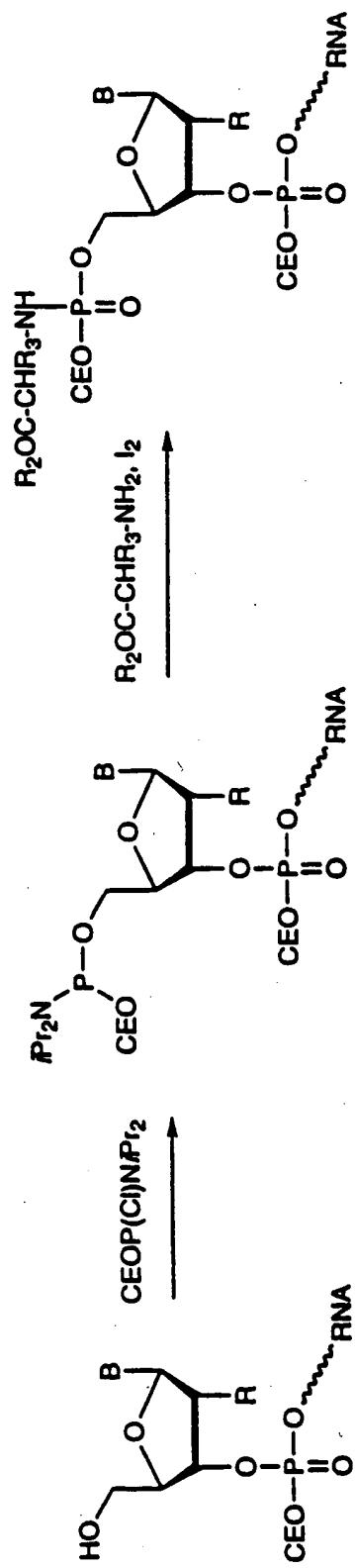
1960-1961

1960-1961

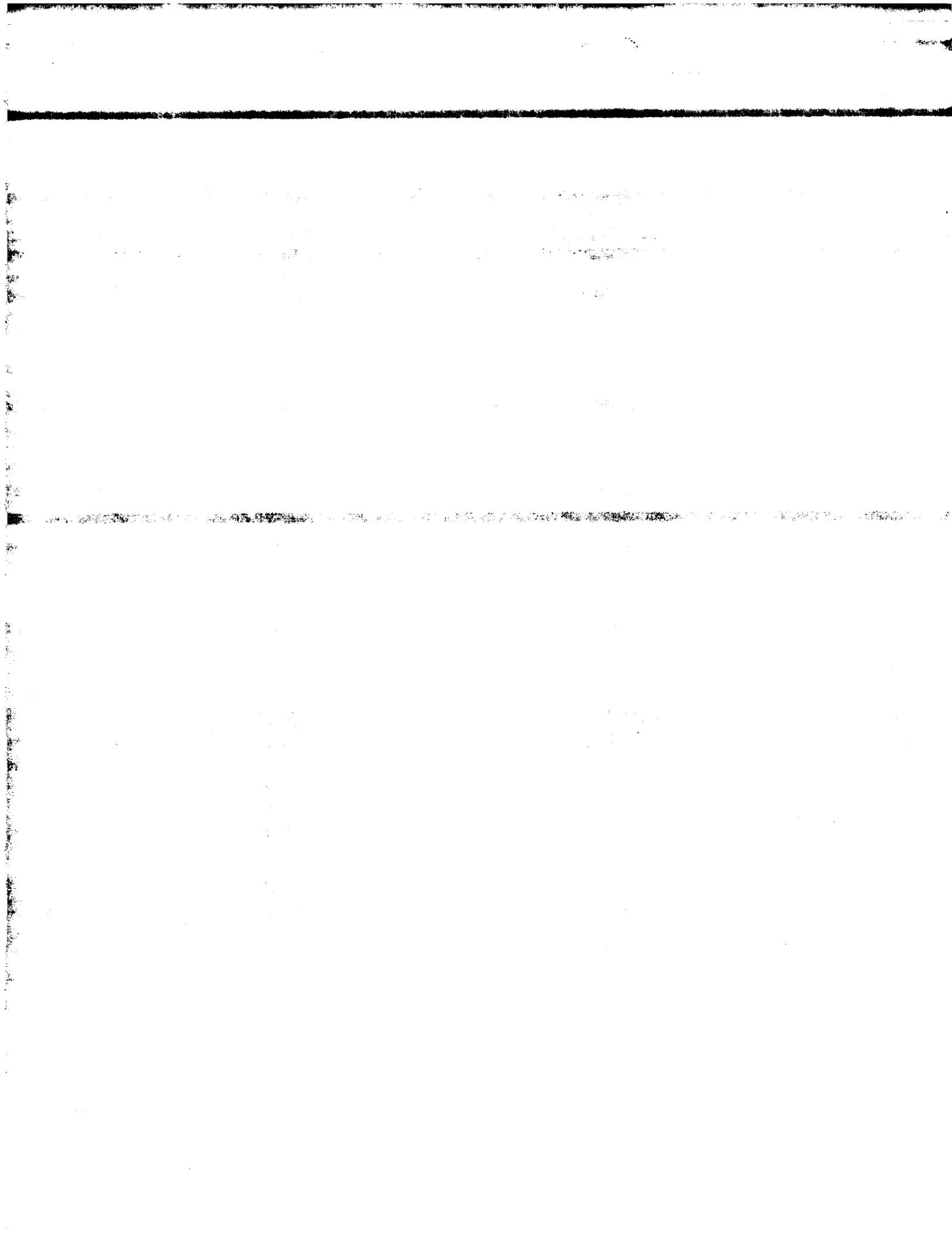
1960-1961

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FIG. 99.



B = UrA, Cyt^{bz}, Ade^{bz}, Gua^{bz}, mod. base, H
 R = H, OCH₃, OTBDMS, HAc, NH*R*₁
 R₂ = OBz, peptidyl



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FIG. 100.

Reversion of mutant RNA

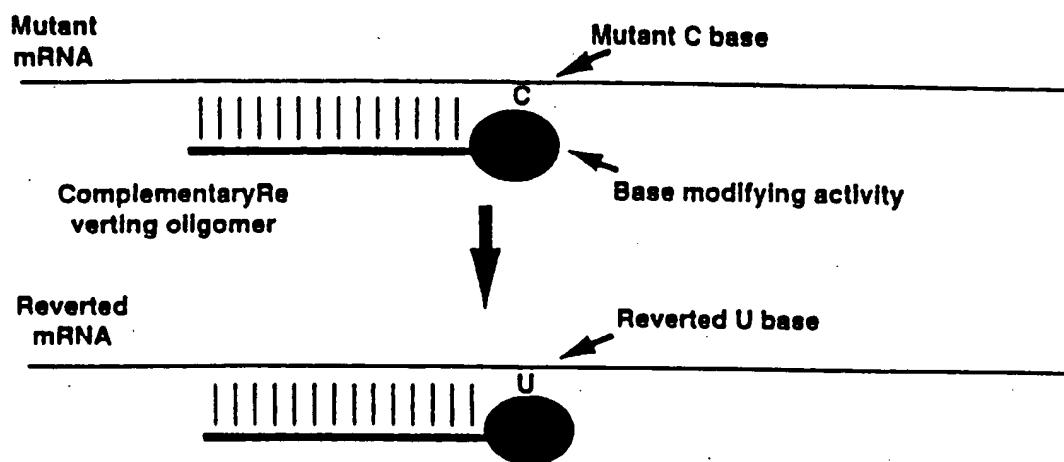
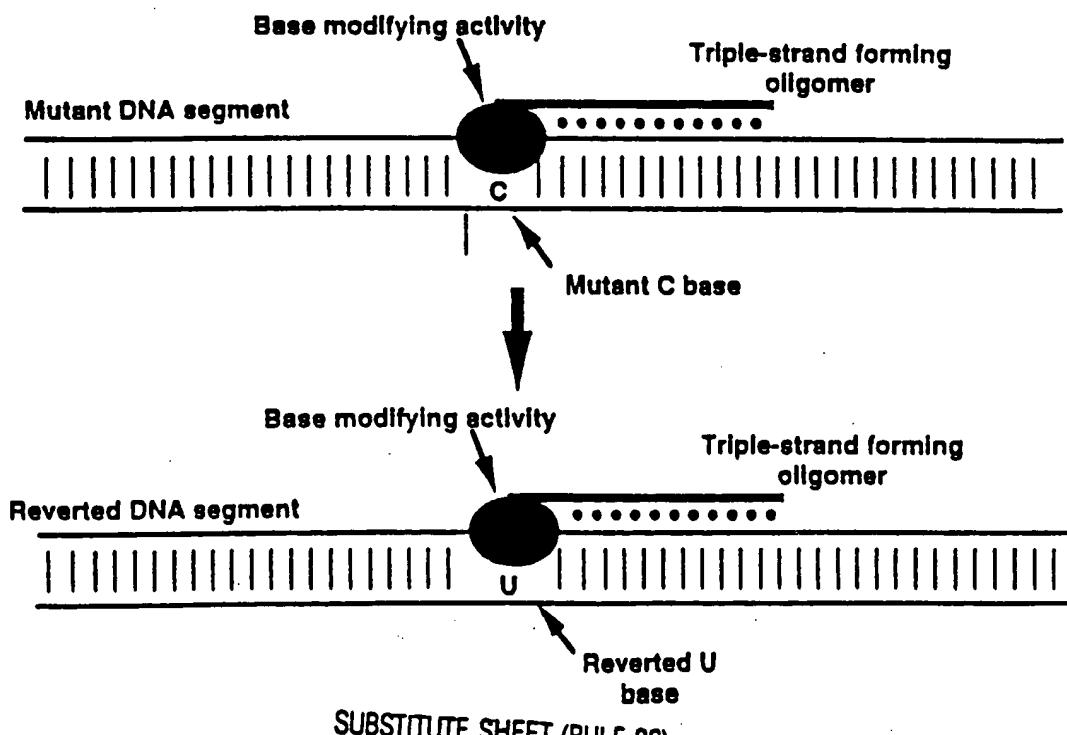


FIG. 101.

Reversion of mutant DNA



SUBSTITUTE SHEET (RULE 26)

Mutant Dystrophin/LUC RNA

Dystrophin segment LUC coding region

UAG
Stop codon mutation

*FIG. 102a.***Target Stop Codon region with Antisense RNA**

Antisense RNA

UAG

*FIG. 102b.***dsRNA Adenine to Inosine unwinding activity**

UIG

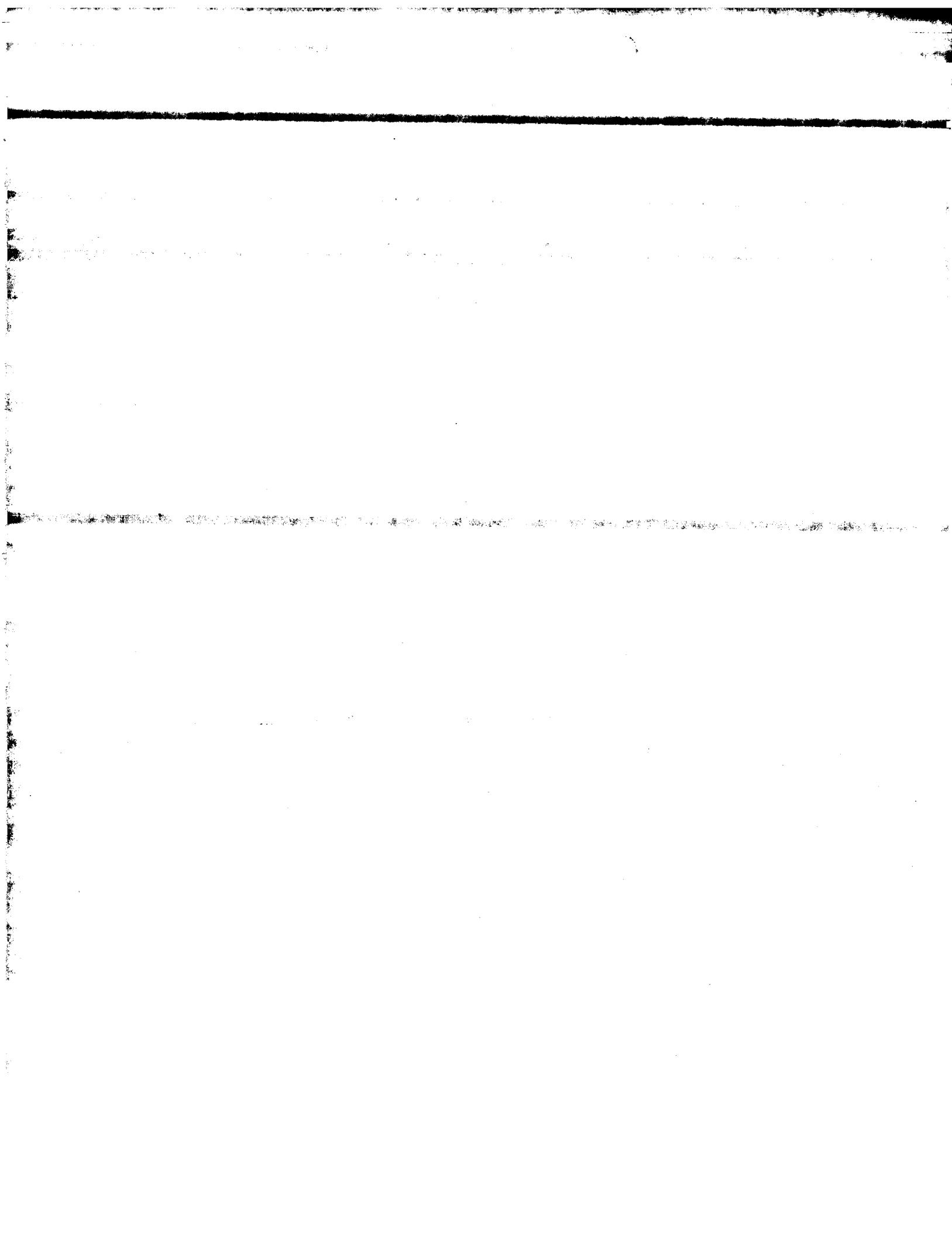
*FIG. 102c.***Translation**

Antisense RNA displaced by ribosome

UIG

R verted stop codon read as UGG
Full length LUC translated

FIG. 102d.



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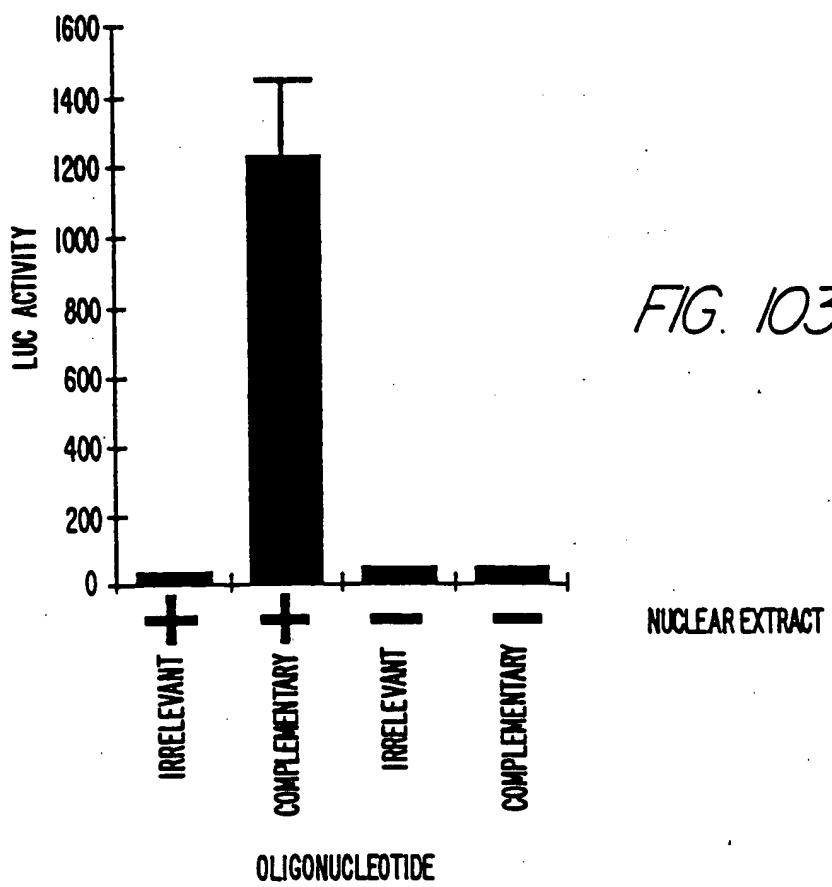
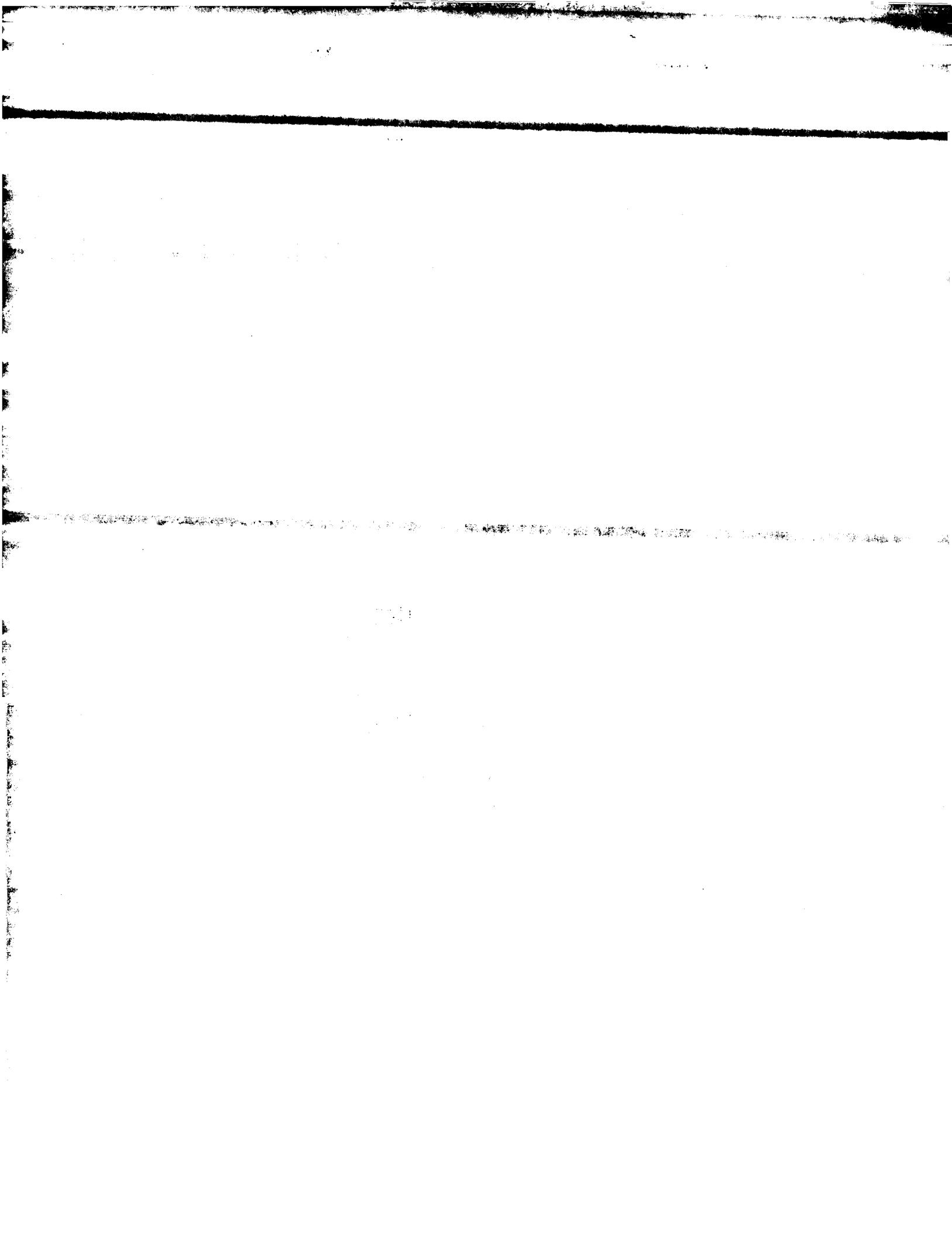
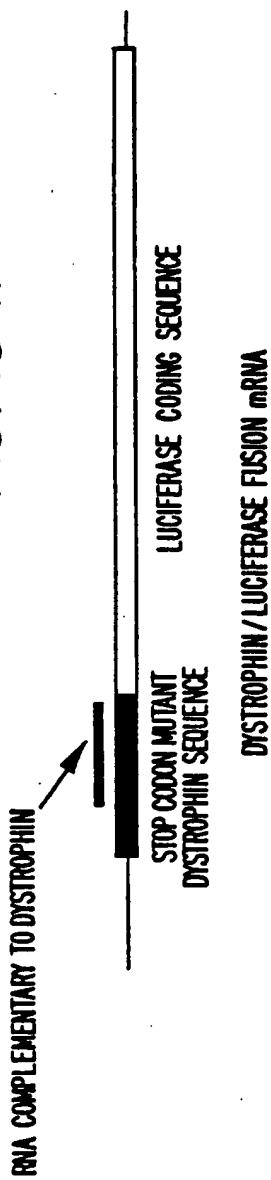


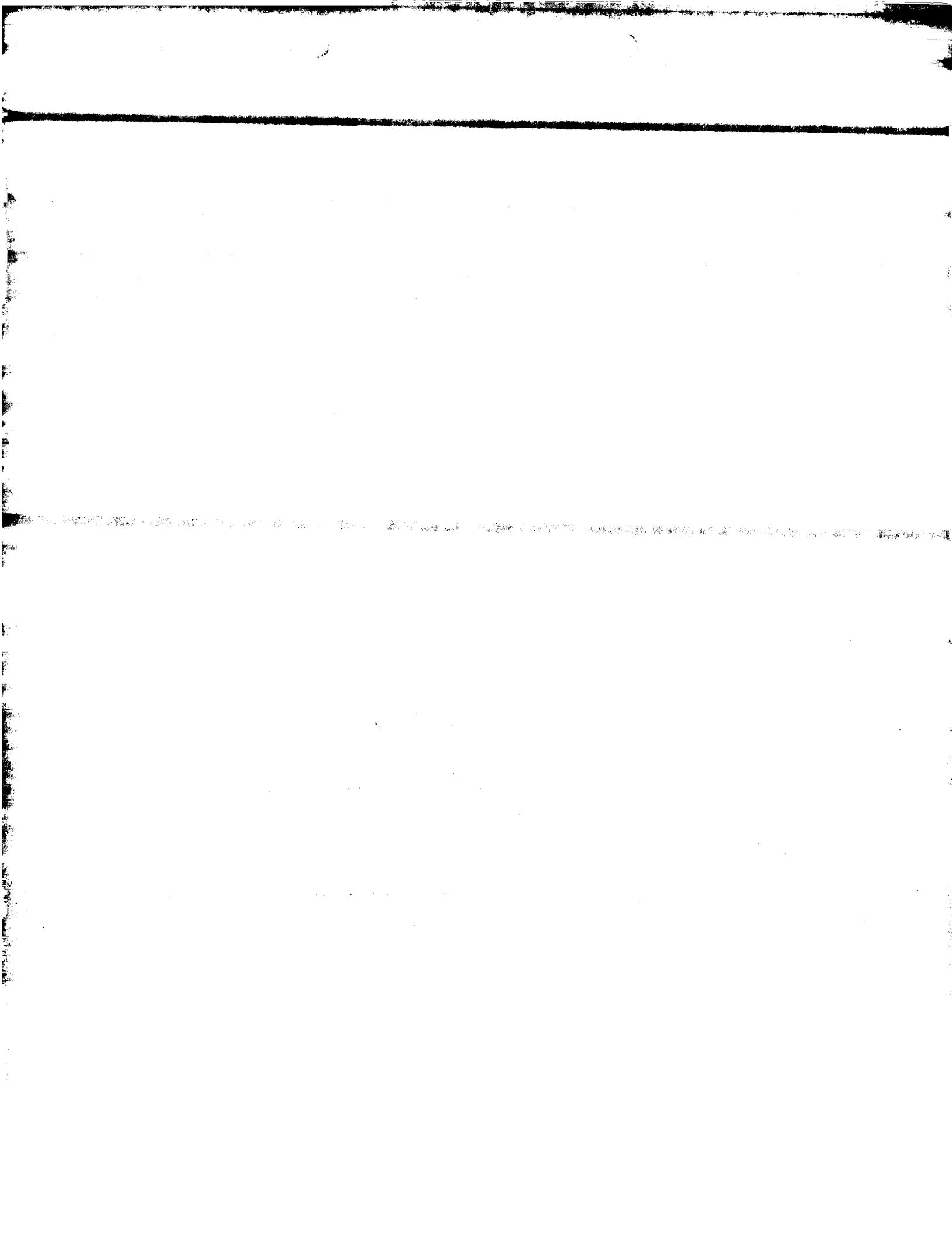
FIG. 103.



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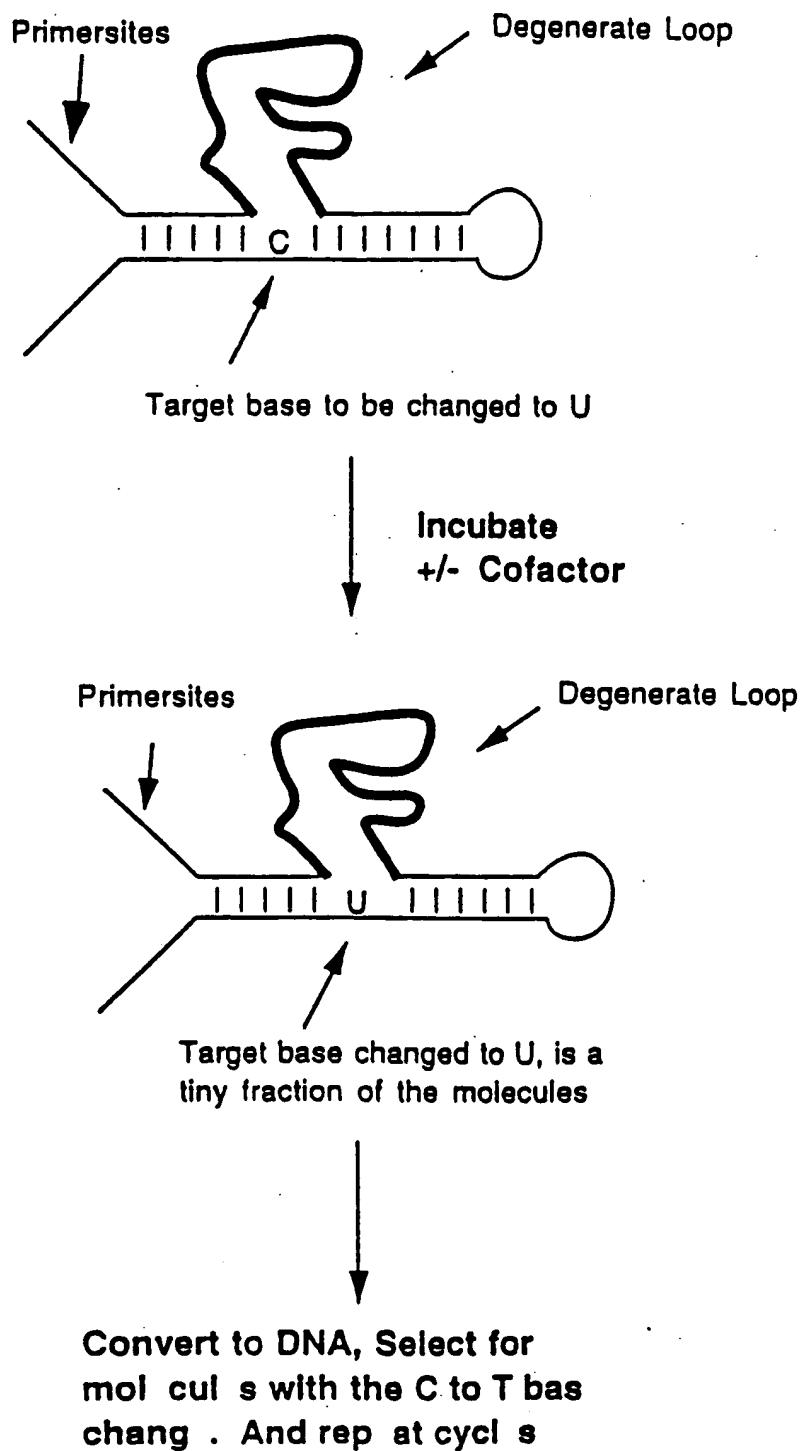
FIG. 104.

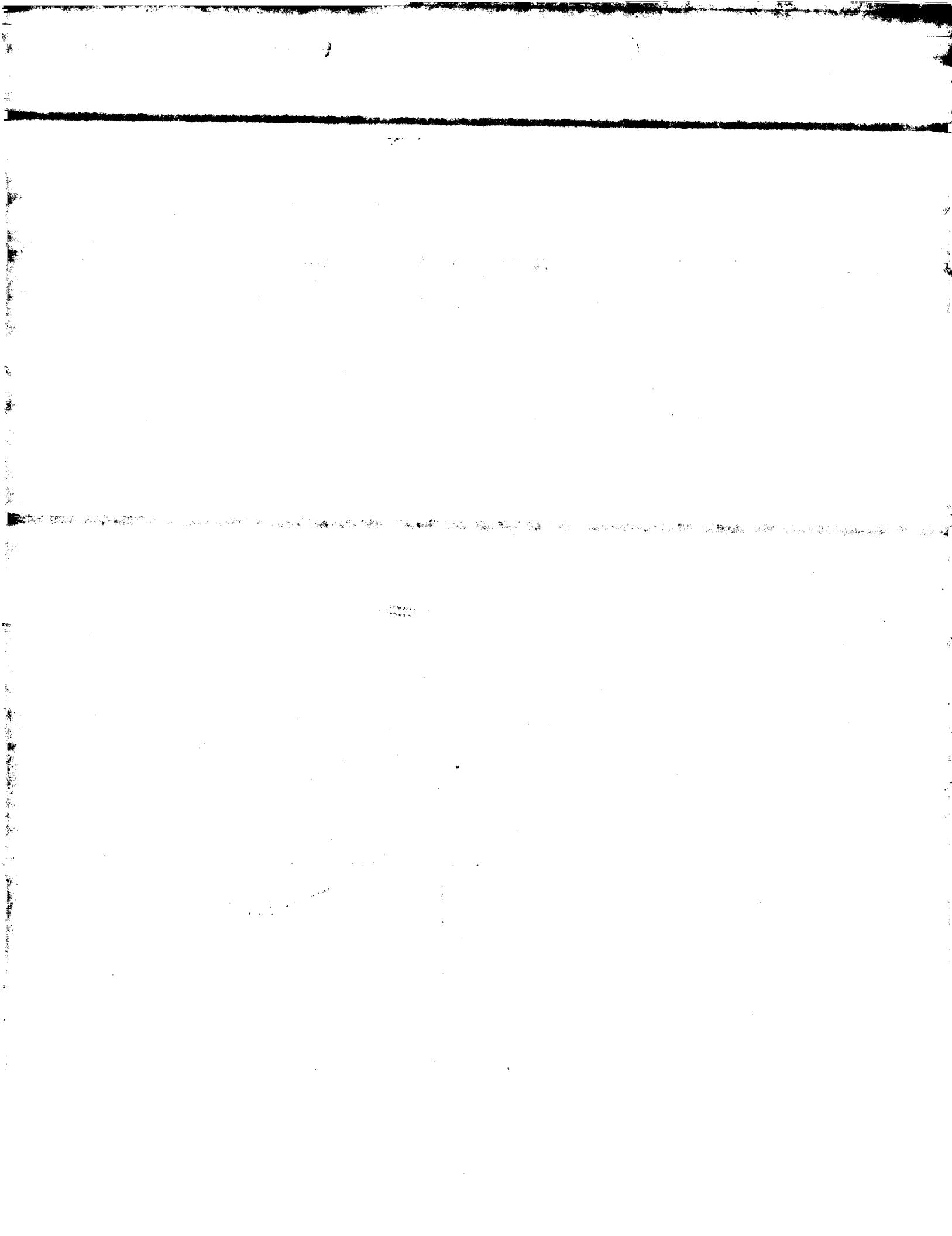




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FIG. 105.





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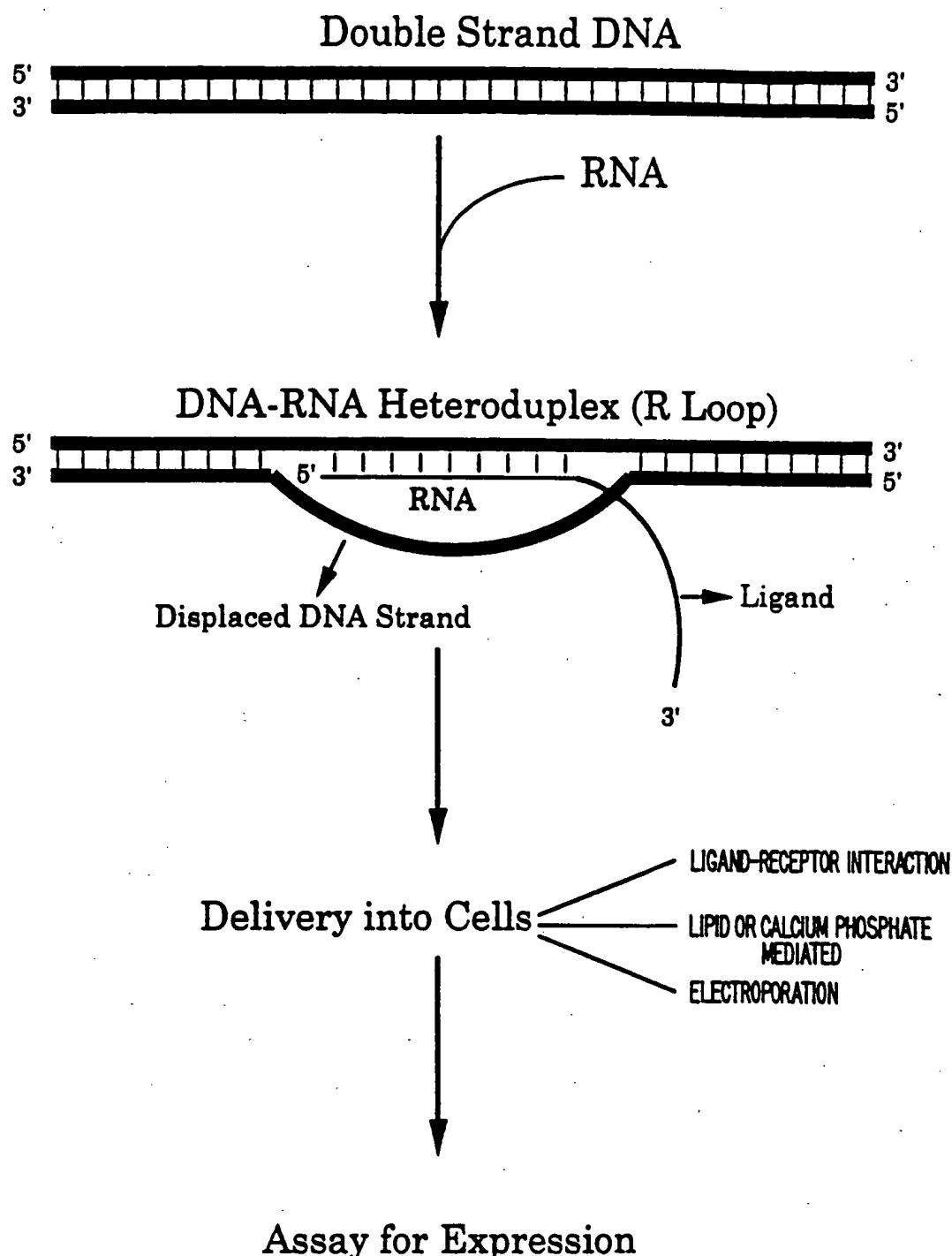
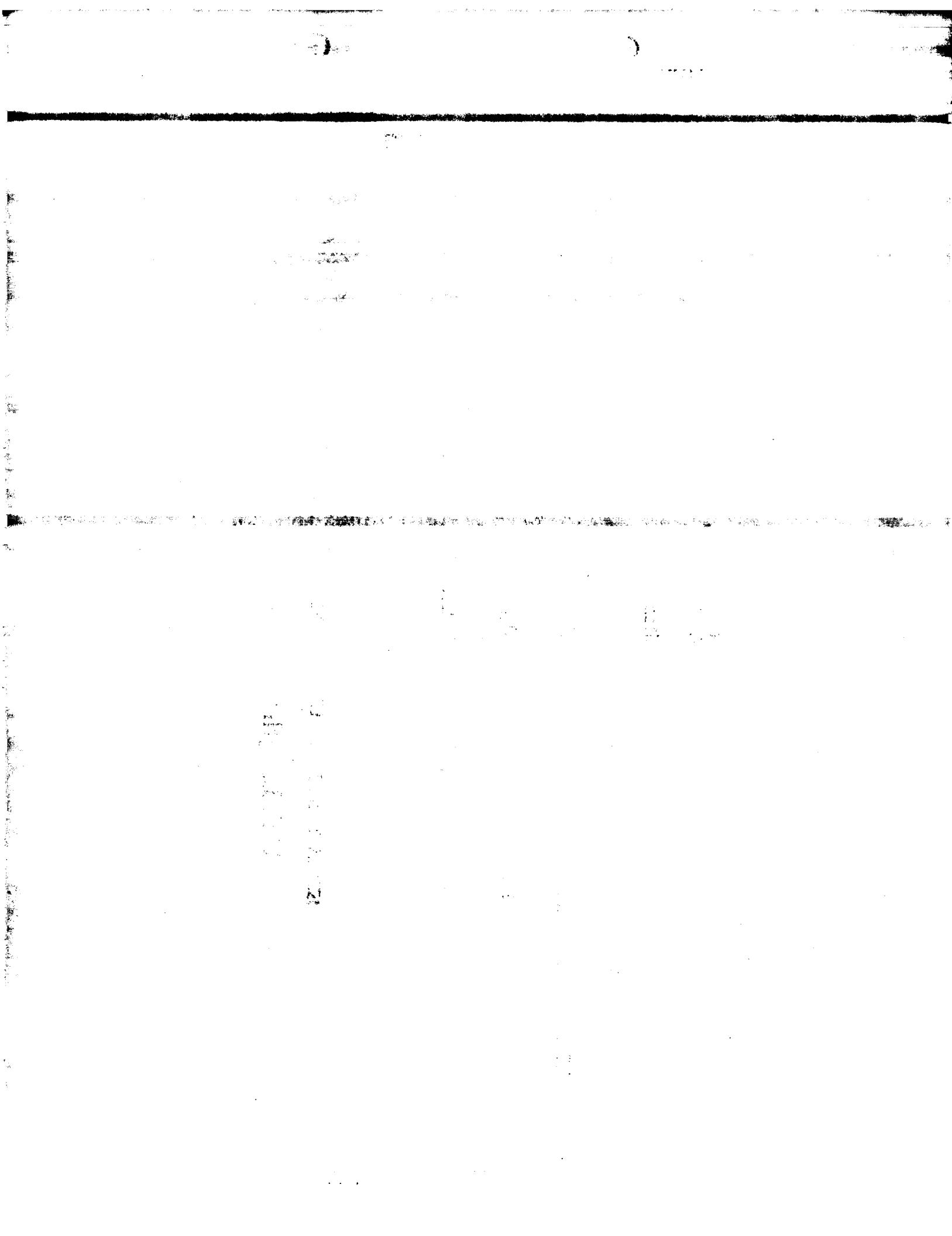


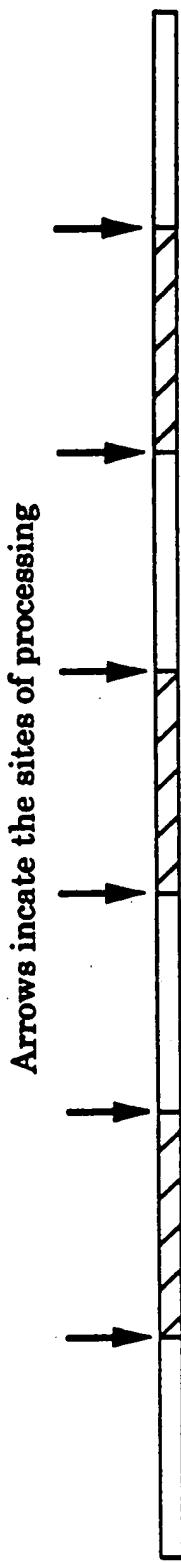
FIG. 106.

SUBSTITUTE SHEET (RULE 26)



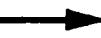
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FIG. 107.



RNA transcript containing multiple ribozyme units

RNA Self-Processing



Unit Length Therapeutic Ribozymes

 → Self-Processing Ribozymes → Therapeutic Ribozymes

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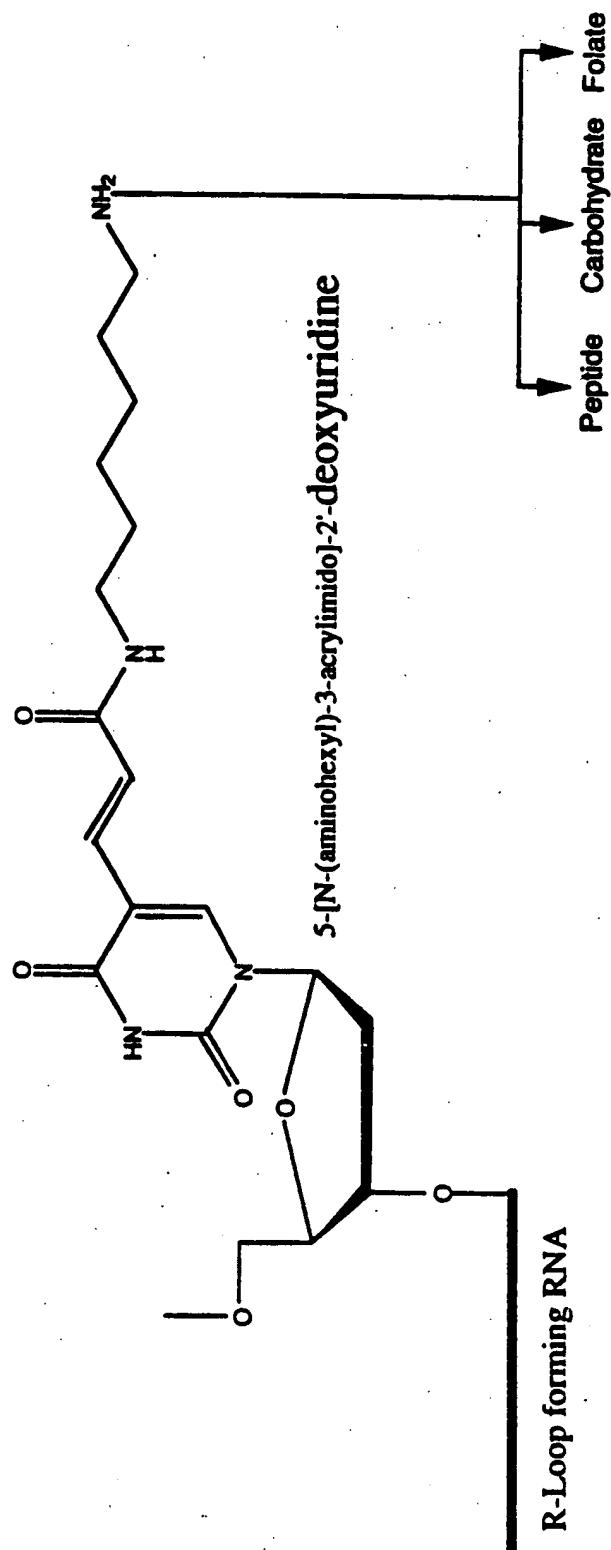


FIG. 108.

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